A SCIENTIFIC EVALUATION OF THE POSSIBLE MECHANISMS OF ANTI – HYPERTENSIVE, DIURETIC AND ANTI-OXIDANT ACTIVITIES OF SIDDHA POLY- HERBAL FORMULATION "*ELADHI CHOORANAM*" IN RODENTS.

The dissertation Submitted by

Dr. R.TAMILSELVAN

Reg. No: 321612110

Under the Guidance of

Dr.M.D. SARAVANA DEVI, M.D.(S).,

Dissertation submitted to

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY

CHENNAI-600032

In partial fulfilment of the requirements

For the award of the degree of

DOCTOR OF MEDICINE (SIDDHA)

BRANCH-II GUNAPADAM

POST GRADUATE DEPARTMENT OF GUNAPADAM

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **A Scientific Evaluation of the Possible Mechanisms of Anti – Hypertensive, Diuretic and Anti-oxidant Activities of Siddha Poly- herbal formulation "*ELADHI CHOORANAM*" in Rodents** is a Bonafide and genuine research work carried out by me under the guidance of **Dr.M.D.Saravanadevi M.D(S).,** Post Graduate Department of Gunapadam, Govt. Siddha Medical College, Arumbakkam, Chennai-106 and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Signature of Candidate**

Place: Chennai **Dr. R. Tamil selvan**

GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **A Scientific Evaluation of the Possible Mechanisms of Anti – Hypertensive, Diuretic and Anti-oxidant Activities of Siddha Poly- herbal formulation "*ELADHI CHOORANAM*" in Rodents** is submitted to the Tamilnadu Dr.M.G.R.Medical University in partial fulfillment of the requirements for the award of degree of M.D (Siddha) is the Bonafide and genuine research work done by **Dr.R.TAMILSELVAN** Under my supervision and guidance and the dissertation has not formed the basis for the award of any Degree, Diploma, Fellowship or other similar title.

Date: **Seal and Signature of the Guide**

Place: Chennai **Dr.M.D.Saravana Devi.MD(S)**

GOVT. SIDDHA MEDICAL COLLEGE,

CHENNAI-106

ENDORSEMENT BY THE HOD AND PRINCIPAL OF THE INSTITUTION

This is to certify that the dissertation entitled **A Scientific Evaluation of the Possible Mechanisms of Anti – Hypertensive, Diuretic and Anti-oxidant Activities of Siddha Poly- herbal formulation "*ELADHI CHOORANAM*" in Rodents** is a Bonafide work carried out by **Dr.R.Tamilselvan** under the guidance of **Dr.M.D.Saravana Devi MD(s),** Post Graduate Department of Gunapadam, Govt.Siddha Medical College, Chennai - 106.

Seal and Signature of the HOD	**Seal and Signature of Principal**
Dr.M.D.Saravana Devi M.D (S)	**Dr.R.Meena kumari M.D (S)**

Date:

Place: Chennai

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CONTENTS

CHART TABLE

ABBREVIATIONS

2K1C	Two kidney one clip
ACE inhibitors	Angiotensin converting enzyme
ALT	Alanine amino transferase
ANOVA	Analysis of variance
ARBs	Angiotensin receptor blockers
AST	Aspartate aminotransferase
Bp	Blood pressure
BUN	Blood urea nitrogen
CCBs	Calcium channel blockers
CCF	Congestive cardiac failure
CMC	Corboxymethylcellulose
CVD	Cardiovascular disease
DMSO	Dimethyl sulfoxide
DOCA	Deoxycorticosterone acetate
DPPH	2,2-diphenyl-1-picrylhydrazyl
EC/ECM	Eladhi chooranam
ECG	Electro cardio gram
EDTA	Ethylenediaminetetraacetic acid
ET	Endothelin
FTIR	Fourier Transform Infra-Red Spectroscopy
GFR	Glomerular filtration rate
GOT	Glutamate oxaloacetate transaminase
GPT	Glutamate pyruvate transaminase
Hb	Haemoglobin
HDL	High density lipoprotein
HPLC	High Performance Liquid Chromatography

IAEC	Institutional animal ethical committee
ICPOES	Inductively coupled plasma optic emission spectroscopy
IHD	Ischemic heart disease
JNC	Joint national committee on prevention, detection , evaluation and treatment of high Blood pressure
LD	Lethal dose
LDL	Low density lipoprotein
MI	Mayocardial infarction
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory drugs
OECD	Organization for economic co-operative development
PCV	Packed cell volume
PVD	Peripheral vascular disease
RBC	Red blood corpuscles
SBP	Systolic blood pressure
SEM	Scanning electron microscope
SEM	Standard error meaning
SHR	Spontaneously hypertensive rats
SLE	Systemic lupus erythematus
TGR(m Ren2)	Transgenic rats over expressing the mouse Ren2
TIA	Transient ischemic attack
TLC/HPTLC	Thin layer chromatography/High performance thin layer chromatography
TSH	Thyroid stimulating hormone
UV	Ultra violet
WBC	White blood corpuscles
WHD	World Hypertension Day
WHL	World Hypertension League
WHO	World health organization

1. INTRODUCTION

So many come to the sickroom thinking of themselves as men of science fighting disease and not as healers with a little knowledge helping nature to get a sick man well

- ***AUKLAND GEDDES***

"Wherever the art of medicine is loved, there is also a love of humanity"

- ***HIPPOCRATES***

Curiosity and the human deserve to understand and influence the environment and to explain and manipulate phenomena has provided the foundations for developing science, philosophy, mythology, religion, anthropology and numerous other fields of knowledge.

Humans initially used to do hunting and fill their craving, later they (embarked/hatched) with agriculture, harvested raw materials are started to brewing. Later they started to cook it in different ways to increase in taste of food. Increase in taste of food decreased the nutrition which need for healthing life and leads to discover many types of disease.

Due to this life span of humans also decreased.

Nowadays scientists are discovering new diseases due to life style modification like change in food habits, living style, sleeping cycle, etc.

In this generation the most leading disease are **Hypertension** and Diabetes, soon Carcinoma will come to this list.

Hypertension is often called the **"SILENT KILLER"**. Hypertension, also known as high or raised blood pressure, is a condition in which the blood vessels have persistently raised pressure. Blood is carried from the heart to all parts of the body in the vessels. Blood pressure is created by the force of the blood pushing against the wall of blood vessels (arteries) as it is pumped by the heart. The highest the pressure the harder the heart has to pump [1].

The word hypertension comes from the prefix “Hyper” meaning “Excessive” and the root word “tension” for “force”. Hypertension literally means “excessive force”. Blood pressure reading consists of two numbers. The higher number is called “systole” and the lower one “diastole”. When the muscles of the heart contract, blood is pumped out to the body.

The Greek word for contraction is “systole”, thus the pressure generated the heart to pump the blood into the circulation is called systolic pressure. The Greek word “diastole” means “drawing apart” and is the term used the phase between heart beats which the muscle relaxes and the heart fills with blood to the pumped out again. The diastolic blood is the pressure in the arteries between beats of the heart.

The normal BP was 120mmHg in systole and 80mmHg in diastole. The first stage of HBP was 130mmHg in systole and the diastolic becomes 80-89mmHg. The second stage ranges from 140 or higher in systolic and 90 of higher in diastolic. One more condition called Hypertensive crisis or Hypertensive emergency, in this condition BP elevated higher than 180mmHg in systolic and the diastolic pressure becomes higher than 120mmHg.[2]

Table No 1: Blood Pressure Levels in Adults

BLOOD PRESSURE CATEGORY	**SYSTOLIC mmHg (upper number)**	**DIASTOLIC mmHg (lower number)**
Normal	Less than 120	Less than 80
Elevated	120-129	Less than 80
High Blood Pressure (Hypertension Stage 1)	130-139	80-89
High Blood Pressure (Hypertension Stage 2)	140 or Higher	90 or Higher
Hypertensive Crisis	Higher than 180	Higher than 120

Table No 2: Ideal BP According to Age

AGE	FEMALE	MALE
1-2 Years	120/75 mmHg	117/76 mmHg
5 Years	104/65 mmHg	103/66 mmHg
10 Years	111/73 mmHg	112/73 mmHg
15 Years	120/76 mmHg	120/78 mmHg
19-24 Years	120/79 mmHg	120/79 mmHg
25-29 Years	120/80 mmHg	121/80 mmHg
30-35 Years	122/81 mmHg	123/82 mmHg
50-55 Years	129/85 mmHg	128/85 mmHg
60+ Years	134/84 mmHg	135/88 mmHg

There are two types of HT. each type has a different cause.

i. Primary HT:

Also called essential HT. it develops over time with no identifiable cause. Most people have this type of HBP. Still unclear what mechanisms cause blood pressure to slowly increase. Factors include Gene, Physical changes and Environmental changes.[3]

ii. Secondary HT:

It often occurs quickly and can become more severe than primary HT. several conditions that may cause secondary HT include Kidney disease, Obstructive sleep apnea, Congenital heart defect, problems with Thyroid, side effects of Medication, Alcohol, Adrenal gland problems, Endocrine tumors. Some with HBP reports Head ache (Particularly at the back of the head and in the morning), as well as light headedness, Vertigo, tinnitus (Buzzing or Hissing in the ears), altered vision or fainting episodes.[4]

These symptoms, however, might be related to associated anxiety rather than the HBP itself. Long term HBP, however, is a major risk factor for Coronary artery disease, Stroke, Peripheral vascular disease, Vision loss, chronic kidney disease and Dementia.[5]

High blood pressure is a major public heart problem in India and its prevalence rapidly increasing among both urban and rural populations. In fact, HT is the most

prevalence chronic disease in India. More than 10 million cases per year. In India, home to more than a sixth of a rapid epidemiological transition. Rates of non-communicable disease have risen in recent decades and are loely to continue as India's population ages and urbanies. Meanwhile, many areas of India still face… infectious disease and poor maternal and child's health.

The prevalence of HT was 20% among women and 24.5% among men. Hypertension in the age group of 18-25 years was 12.1%. The researchers used health data collected from 1.3 million adults across India.

Hypertension among middle aged adults in the poorest households in rural areas was also high 30% had HT. Rates of HT is high among people geographic measures and sociodemographic groups in India.

The prevalence of HT ranges from 20-40% in urban adults and 12-17% among rural adults. The number of people with HT is projected to increase from 1.8 million in 2000 to 214 million in 2025, with equal numbers of men and women.[6]

A survey of 26000 adults in south India showed a hypertension prevalence of 20% (Men 23% Women 17%) but 67% of those with HT were unaware of their diagnosis.[7]

Prevalence of HT in urban is 22-30%. The latest study gives importance as there is very little on prevalence of HT in rural Tamilnadu.

Hypertension is one of the major risk factors for CVD, Stroke and Kidney failure. About 24.5% of deaths in people aged 45-59 years in rural Tamilnadu are caused by diseases of the circulatory system problems.[8]

Hypertension management deals not only in reducing the blood pressure but also minimize the cardiovascular risk by lifestyle measures, lipid managements, smoking cessation, dietary intervention, weight reduction and physical activity. Uncontrolled blood pressure can lead to stroke, aneurysm, heart failure, vision loss, metabolic syndrome and even memory loss.[9]

A variety of blood pressure lowering medicines (Antihypertensive drugs) is available in market.

1. Angiotensin Converting Enzyme
2. Angiotensin II receptor blockers
3. Calcium channel blockers
4. Diuretics
5. Beta-blockers
6. Alpha-blockers
7. Centrally acting Anti-hypertensive drugs
8. Vasodilators which reduce BP by dilatation of blood vessels Eg: Hydralazine.

Adverse effect of these drugs:

Dry cough, Dizziness, Taste disturbances and Rashes, Head ache, Dizziness, flushed face, Head ache, swollen ankles, Constipation, Dizziness and Tiredness, Urinary frequency, Dizziness, Gastro intestinal disturbances, Tiredness, Cold hands and feet, Slow heartbeat, Diarrhoea and Nausea, Sleep disturbances, Nightmares, Hypotension (notably postural Hypotension), Syncope, Asthenia, and Tachycardia.[10]

India's ancient vogue and Tamilnadu conventional medicine was that the *Siddhars* devoted *Siddha* medicine.

"Natural forces within us are the true healers of disease"

- ***HIPPOCRATES***

Siddha means Knowledge. *Siddhars* are Spiritual scientists. *Siddha* medicine not only cures the physical illness but also for the soul illness.

Siddha is one of the earliest traditional medicine systems in the world which treats not only the body but also the mind and the soul. The word Siddha has its origin in the Tamil word Siddhi which means "an object to be attained" or "perfection" or "heavenly bliss". India being the birth place of many traditional philosophies also gave birth to Siddha. The roots of this system are intertwined with the culture of ancient Tamil civilization.

"*Siddhargal*" or *Siddhars* were the premier scholars of this system in ancient times, *Siddhars*, mainly hailing from Tamil Nadu laid the foundation for Siddha system of medicine. *Siddhar*s were spiritual masters who possessed the asta (eight) Siddhis or unique powers. *Agasthiyar* is believed to be the founding father of siddha medicine. Eighteen Siddhars are considered to be pillars of Siddha medicine. Siddha medicine is clamied to revitalize and rejuvenate dysfunctional organs that cause the disease. Kayakarpam, a special combination of medicine and life style, Varmam theray, Vaasi (Pranayamam) and Muppu the universal salt are the specialities of *Siddha* system of medicine. Thus this system connects both spiritual and physical and treats the person as a whole i.e. it concentrates the physical, psychological, social and spiritual well-being of an individual.

"Healing is a matter of time, but it is sometimes also a matter of opportunity"

- ***HIPPOCRATES***

According to *Siddhars*, human body is made up of *Pancha boothas* (*மண், நீர், தீ, வளி, வெளி),* Three humors *(வாதம், பித்தம், கபம்),* Seven *Udarkatugal (சாரம், செந்நீர், ஊன், கொழுப்பு, என்பு, மூளை, சுக்கிலம்).*

'The environment is not separate from ourselves.

We are inside it, and it is inside us.

We make it, and it makes us.'

- **YANOMAMI**

Cosmos energy is present with in the human body also. If there changes in the cosmos it also changes in the human body. This well said as

"அண்டத்தில் உள்ளதே பிண்டம்

பிண்டத்தில் உள்ளதே அண்டம்"

மறுப்பது உடல் நோய் மருந்தெனலாகும்

மறுப்பது உள நோய் மருந்தெனச்சாலும்

மறுப்பது இனி நோய் வாராதிருக்க

மறுப்பது சாவை மருந்தெனலாமே"

\- *திருமந்திரம்*

One that cures physical ailment is medicine

One that cures psychological ailments

One that prevents ailment is medicine

One that bestows immortality is medicine.

– ''Thirumandhiram"

The above verses mean that the medicine should be improve as a curative, preventive form and should be significantly improve the quality of life duration said by 'Thirumoolar'. That kind of medicines are found in our system to treat high blood pressure but is not scientifically validated.

Here to overcome this serious consequence of Hypertension we need proper medication and improved quality of life. Hence, in the treatment aspect of *Siddha* system the present investigations decided to choose the polyherbal formulation of ***Eladhi chooranam***. I hope this formulation of polyherbal trail drug will be effective in the management of hypertension after preclinical validation of Anti-Hypertensive, Diuretic, Anti-Oxidant activity.

2. AIM AND OBJECTIVES

AIM:

The aim of this study is to validate the **Anti-hypertensive, Diuretic and Anti -oxidant** Activity of ***Eladhi chooranam*** and thus ensuring a holistic approach by controlling the blood pressure level, significantly decreasing the development and progression of complication of *Kuruthi Azhal Noi* (HT)

OBJECTIVES:

The main objective of the present study is to highlight the safety and efficacy of *Eladhi chooranam* in the treatment of *Kuruthi Azhal Noi*, the following methodology was adopted to evaluate the drug and standardization studies.

The key objectives of the study are:

- Collection of various Siddha and modern literature relevant to the study.
- Preparing the drug according to Siddha classical text.
- Subjecting the drug into Physico-chemical standardization.
- Analyzing the drug chemically for detection of acid and basic radicals.
- Focusing the drug for analytical assessment through sophisticated analytical modern techniques like FTIR, ICP-MS, SEM, XRD, GCR.
- Studying the toxicity profile of *Eladhi chooranam* according to OECD guidelines.
- Evaluation of the pharmacological activity of the test drug *Eladhi chooranam* through the following activities,

  Anti-hypertensive, Diuretic Activities in wistar albino rats

  Anti- Oxidant activity - Through DPPH assay
- Evaluation of Microbial load for this formulation.
- Analyzing all the above study results to evaluate the benefits of ***Eladhi Chooranam***

3. REVIEW OF LITERATURE

DRUG REVIEW:

ELADHI CHOORANAM

ஏலமில வங்கந்தா மரைநற் பூவி

னிதழிலந்தைக் கொட்டையுட பருப்பும் நல்ல

கோலமுய ரல்லிமல ரிதமும் வாசங்

குலவுபச்சைக் கர்ப்பூர முத்தக் காசு

சீலநெல்லின் பொரியுமாதி மதுர மொப்பாய்ச்

செய்து பொடி நார்கொருக ழஞ்சு தேனில்

மேலதனைக் கூட்டியுண்டா லிரத்த பித்த

மிதமில்லாப் பெரும்பாடும் வாசி யாமே.

(108)

Ingredients:

- *Elakkai (Elettaria cardamomum)*
- *Thamarai poovithaz (Nelumbo nucifera)*
- *Allipoo (Nymphaea nouchali)*
- *Korai kizhangu (Cyperus rotundus)*
- *Athimathuram (Glycyrrhiza glabra)*
- *Kirambu (Syzygium aromaticum)*
- *Ilanthai kottai paruppu (Ziziphus mauritiania)*
- *Pachai karpooram (Dryobalanops aromatica)*
- *Nerpori (Oryza sativa)*

3.1 GUNAPADAM ASPECT OF THE TRIAL DRUG [11]

Elakkai (Elettaria cardamomum)

Other names: *Anji, Korangam, Thudi.*

Vernacular names:

English **:** Cardamomum seeds

Telugu **:** Elakulu

Malayalam **:** Elattari

Hindi **:** Elachi

Sanskrit **:** Ela

Part used **:** Seeds

Properties:

Taste : Acrid

Character: Hot

Division : Acrid

Actions:

- Carminative
- Stimulant
- Stomachic

General character:

"தொண்டைவாய்கவுள் தாலுகு தங்களில்
தோன்றும் நோயதி சாரம்பன் மேகத்தால்
உண்டை போல்எழுங் கட்டி கிரிச்சரம்
உழலை வாந்தி சிலந்தி விஷஞ்சுரம்

பண்டை வெக்கை விதாகநோய் காசமும்
பாழுஞ் சோமப் பிணிவிந்து நட்டமும்
அண்டை யீளைவன் பித்தம் இவைக்கெல்லாம்
ஆல மாங்கமழ் ஏலமருந்ததே[11a].

-தேரன் குணவாகடம்

ஏலம்

……..

……புண்டரிக

நாலுதிர மூக்கு நலிகேல நாவியொடு

நாலுதிர மூக்குபத்து நாள்.[11b]

Indications:

- It cures Cough, Dysuria, Dysentery and it increases the sperm count.
- The powder of *E.cardamonum* which is used as *Akiranam* and cures epistasis, sinusitis, headache.

Therapeutic Uses:

- *Ela vadakam* used to cure stomach pain, diarrhoea.
- *Ela nei* cures thirst, disorders in the stomach and cools the body and increases the blood.
- Decoction of Elam, which cures *Thaba suram.*

Thamarai poovithazgal (Nelumbo nucifera)

Other names:

Raaseevam, Irumbu, Maraipoo, Sooriya natpu, Aravindham, Kamalam, Pundarikam, Pathumam,.

Vernacular names:

English **:** The sacred Lotus

Telugu **:** Tamara

Malayalam **:** Aravindam

Sanskrit **:** Pankaja

Part used **:** Flower, Petals, Seed, Rhizome

Properties:

Taste **:** Sweet, Astringent

Character: Coolant

Division **:** Sweet

Action:

- Coolant
- Astringent
- Expectorant
- Sedative

General character:

பருத்தநற் றாமரைப்பூ பல்வாந்தி நோயைத்
துரத்திவிடும் இன்னுஞ் சொல்லவோ-கரத்தில்
எடுத்தணைக்க்க் கண்குளிரும் ஏகுஞ் சுரமும்
எடுத்தவி தாகமும்போம் எண்.[11c]

-அகத்தியர் குணவாகடம்

தாமரைப் பூத்தாள்

"சண்டனையுஞ் சண்டனையுந் தள்ளமல ருள்ளுறையுண்
சண்டனையுஞ் சண்டலையுஞ் சார்".[11d]

- *தேரையர் யமகம்*

Indications:

- It cures abdominal pain, Itching, Thirst, Fever, Heart diseases.
- It cures eye irritation due to body heat.

Therapeutic uses:

- Pollen powder of Lotus+Sugar+Honey mixed well and which cures Deafness and improves Aphrodisiac activity.

Alli poovithazgal (Nymphaea nouchali)

Other names:

Album, Kumutham, Kairavam.

Vernacular name:

English **:** Water lilly

Telugu **:** Kalava

Malayalam **:** Allit-tamara

Sanskrit **:** Kumudam

Hindi **:** Kanava

Part used **:** Flower, leaf, Rhizome

Properties:

Taste **:** Astringent

Character **:** Coolant

Division **:** Sweet

Actions:

- Emollient

General character:

செவ்வல்லிப் பூவுக்குக் சேர்ந்திறங்கு நீர்ப்பிணியோ
டொவ்வுமே கப்பிணியும் ஓய்வதன்றி-இவ்வுலகிற்
கண்ணின்நோய் தீரும் கனத்தபித்த ரத்தமொடு
புண்ணின்நோய்பன்னோயும் போம்.[11e)]

-அகத்தியர் குணப்பாடம்.

Indications:

- It cures Diabetes, Hypertension, Eye disorders due to over body heat, Wounds, Venereal disease, Urinary tract infection, thirst, and body heat.
- The leaf decoction used to wash wounds.

Korai kizhangu (Cyperus rotendus)

Other names:

Muthakaasu.

Vernacular name:

English **:** Nut grass

Telugu **:** Tungamuste

Malayalam **:** Muththanna

Part used: Rhizome

Actions:

- Diuretic
- Diaphoretic
- Emmenagogue
- Stimulant
- Tonic
- Demulcent

General character:

சீத சுரந்தீர்க்குஞ் செம்புனல்பித் தம்போகும்
வாத சுரந்தணிக்கும் வையகத்தில்-வேதைசெய்ய
வந்த பிணியையெல்லாம் வாட்டுமுத் தக்காசு
கொந்துலவும் வார்குழலே! கூறு
அதிசாரம் பித்தம் அனற்றாகம் ஐயங்
குதிவாதஞ் சோபங் கொடிய-முதிர்வாந்தி
யாரைத் தொடர்ந்தாலும் அவ்வ்வர்க்கெ லாங்குளத்துக்
கோரைக் கிழாங்கை கொடு [11f]

-அகத்தியர் குணவாகடம்

Indications:

- It cures Hypertension, Thirst, Delirium and Vomiting**.**
- Decoction cures Diarrhoea and Ulcer.
- While applying the paste externally, it is used to treat scorpion sting bite.

Therapeutic uses:

"கோல வுணவைக் குமர னடலிலடு
கோல வுணவைக் கொடுக யத்தை" [11g]

- It is used in the treatment of Tuberculosis.
- Sleeping in Korai mat causes cooling effect in body and sleep.

Athimaduram (Glycyrrhiza glabra)

Other names: *Athingam, atti madhugam, Kundri ver.*

Vernacular names:

English **:** Jequitity, Indian or Jamaica liquorice

Telugu **:** Ati-Madhuramu, Yasti-Madhukam

Malayalam **:** Ati-Madhuram, Iratti-Madhurarr

Sanskrit **:** Yashti-Madhukam

Hindi **:** Jathi-Madh, Mulath

Part used: Root

Properties:

Taste **:** Sweet

Character **:** coolant

Division **:** Sweet

Actions:

- Emollient
- Demulcent
- Expectorant
- Laxative
- Tonic

General character:

கத்தியரி முப்பிணியால் வருபுண் தாகங்

கண்ணோய் உன்மாதம்விக்கல் வலிவெண் குட்டம்

பித்தமெலும் புருக்கி கிரிச்சரம் ஆவர்த்த

பித்தமத மூர்ச்சை விட பாகம் வெப்பந்

தத்திவரு வாதசோ ணிதங்கா மாலை

சருவவிடங் காமியநோய் தாது நட்டங்

குத்திருமல் ஆசியங்கம் இதழ்நோய் இந்து

குயப்புணும்போம் மதுரகமெனக் கூறுங் காலே[11h].

- *தேரன் குணவாகடம்.*

Indications:

- It is also indicated for Jaundice, Arthritis, Eye diseases, Skin diseases, Leukoderma and Migraine.
- Used to cure burning sensation, removes body toxins and cures psychiatric diseases.

Therapeutic uses:

- The root of Indian liquorice is chewed for cough.
- The legium of Indian liquorice cures burning mituration.
- Indian liquore powder cures head ache, migraine, fever and improves eye site.

Kirambu (Zysygium aromaticum)

Other names:

Anjukam, Urkadam, karuvaai kirambu, Sosam, Thirali, varaankam.

Vernacular name:

English **:** Clove

Telugu **:** Lavangalu

Malayalam **:** Karampu

Sanskrit **:** Lavangam

Hindi **:** Long

Part used: Leaves, Flower

Properties:

Taste **:** Sweet, Acrid

Character **:** Hot

Division **:** Acrid

Action:

- Antispasmodic
- Carminative
- Stomachic

General characters:

பித்த மயக்கம் பேதியொடு வாந்தியும் போம்

சுத்தவிரத் தக்கடுப்புந் தோன்றுமோ-மெத்த

இலவங்கங் கொண்டவருக் கேற் சுகமாகும்

மலமங்கே கட்டுமென வாழ்த்து.

சுக்கிலனட் டங்கர்ண சூர்வியங்க லாஞ்சன்ந்தாட்

சிக்கல்விடாச் சர்வா சியப்பிணியு-மக்கிக்குட்

டங்கப் பூவோடு தரிபடருந் தோன்றிலி

வங்கப்பூ வோடுரைத்து வா [11i].

- *அகத்தியர் குணவாகடம்*

Uses:

- It cures prolonged Diarrhoea, Dysentery, Ear diseases and Tinea infections.
- This is used as cooking masala.

Therapeutic uses:

- The clove powder decoction stops hyperemesis gravidarum.
- Increases appetite and promotes digestion.
- Clove oil Externally act as
    a. Antiseptic
    b. Local anaesthetic
    c. Rubefacient
- Internally act as
    a. Stomachic
    b. Nutritive
- Dip the cotton balls in the clove oil and placed it over the upper and lower jaw which cures tooth ache.

Ilandhai kottai paruppu (Ziziphus mauritiania)

Other names:

Kullathi, Kulvali, Kol, Korkodi, Vathari.

Vernacular name:

English **:** The Indian jujube tree, Chinese dale

Telugu **:** Regu

Malayalam **:** Ilanda

Sanskrit **:** Badari; Kola

Hindi **:** Baer

Part used: Leaves, Fruit, Bark, Root, Root barks, Wood, Lentil nut, Whole plant,

Properties:

Taste **:** Sour, Sweet

Character **:** Coolant

Division **:** Sweet

Action:

- Astringent
- Emollient

General Character:

கொண்டையா மிலந்தையைக் கொள்ளவேத்திறத்திலும்
மண்டிய நோயெலா மாய்ந்துபோய் விடுமே. [11j]

Indications:

- It cures Pitha diseases
- Decoction of leaf and bark cures dysentry and abdominal cramp.

Nerpori (Oryza sativa)

Other names:

Thorai, Vai, Viriki, Sen nel, Saali, Vari.

Vernacular name:

English **:** Paddy

Telugu **:** Vari

Malayalam **:** Nella

Sanskrit **:** Vrihi

Hindi **:** Chaval

Part used: Rice, Bran, Husky

Properties:

Taste **:** Sweet

Character **:** Coolant

Division **:** Sweet

Action:

- ❖ Nutrient

General Character:

நெற்பொரியைத் தின்றால் நெடுந்தாகம் வாந்திமந்தம்

மற்பித்த வாதமத மூர்ச்சை-பற்பலவாம்

பேதி யருசியிவை விட்டொழியுஞ்

சாதி மடமயிலே சாற்று[11k]

- அகத்தியர் குணவாகடம்

Indication:

- It cures abdominal pain, leucorrhoea, burning micturation, fever and gives strength to those patients.
- And also which cures thirst, tastelessness, ascites, indigestion, vomiting.

Therapeutic uses:

- Decoction of *Nerpori* cures prolonged vomiting and ulcer.

Pachchai karpooram (Drobalanobs aromatica)

One among the twenty five type of Karasaaram.

Other names:

Imavalugam, Kathaliuppu, Kelithipachchai, Sasi, Chandran, Somanuppu, Seethalam, Mathi, Maruvaali.

General properties:

"அட்டகுன்மஞ் சூலை யணுகாது வாதமொடு

துட்டமே கபபிணியுந் தோற்றாதே - மட்டலருங்

கூந்தலுடை மாதே கொடியகபம் போகுஞ்

சார்பச்சைக் கர்ப்பூரத் தால்." (111)

- *அகத்தியர் குணவாகடம்*

Effective in eight types of gastric ulcers, *Vatha* disease, joint pain and *Kapha*.

Action:

- Expectorant
- Tonic
- Demulcent

Uses

- Scabies, bad odour due to excess sweating.
- It controls leucorrhoea as an internal medicine.
- Along with musk gives stimulant action and cures fever due to *Kapha Vatha*, cough, asthma, pulmonary diseases.
- Mixed with sandal paste and applied over the body for demulcent effect.

3.2 BOTANICAL ASPECT:

Elettaria cardamomum [12]

Taxonomical classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Liliopsida

Order : Zingiberales

Family : Zingiberaceae

Genus : *Elettaria*

Species : *cardamomum*

Elettaria cardamomum

Distribution:

Throughout in India.

Description:

Stem perennial, erect, joined, 6-9 feet, enveloped in the sheaths of leaves; leaves lanceolate, acuminate, sub-sessile, entire, 1-2 feet long; sheaths slightly villous; scapes several, flexuose, joined, branched, 1-2 feet long; flowers alternate, short stalked, solitary at each point of the raceme; calyx funnel shaped, 3-toothed, inely striated, corolla tube as long as the calyx; limb doubled exterior portion of 3 oblong, concave, nearly equal division; inner lip obovate, longer then the exterior division, curled at the marings.

Apex 3- lobed, marked in the centre with purple white stripes; capsule oval, somewhat 3-sided, 3- celled, 3-valved; seeds numerous, angular; flowers pale-greenish white.

Part used: Seeds

Chemical constituents:

α-pinene, β-pinene, sabinene, myecene, a-phyllandrene, limonene; 1,8-cineole, y-terpinene, p-cymene, terpinolene, linalool, linalyl acetate, terpinen-4-oil, α-terpineol, α-terpineol acetate, citronellol, nerol, geraniol, methyl eugenol and trans-nerolidol.

Properties and Uses:

As cordial and stimulant the seeds are frequently used medicinally, but more frequently as corrective in conjunction with other medicines. A volatile is produced from them by distillation, which has a strong aromatic taste, soluble in alcohol. It loses its odour and taste by being kept too long. The natives chew the fruit with betle, and use it in decoction for bowel-complaints and to check vomiting in infusion it is given in cough.

Nelumbo nucifera [13]

Taxonomical classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Proteales

Family : Nelumbonaceae

Genus : *Nelumbo*

Species: *nucifera*

Nelumbo nucifera

Distribution:

Throughout in India, in marshes and ponds upto an elevation of 1,800m.

Description:

A large handsome aquatic herb with slender elongate, branched, creeping, rhizomes sending out roots at the nodes; leaves pelate, 60-90 cm or more in diameter; petioles very long, smoother or with small prickles, much raised out of water; flower solitary large, fragrant, white or rosy with a centrally located yellow obconical spongy tours in which capsules are shrunken; fruits ovoid, nut like achenes.

Part used: Whole plant

Chemical constituents:

Linalool, nonadecane, phytol, raffinose, neferine, nelumbine, liensinine, isoliensinine, nuciferine.

Properties and uses:

- The plant is astringent, bitter, sweet, cooling, emollient, diuretic, anti-fungal, anti-pyretic, cardiactonic. It is useful in hyperdipsia, vitiated conditions of pitta, cholera, diarrhoea, helminthiasis, vomiting, burning sensation, haemorrhoids, nervous exhaustion, ringworm, dermopathy, intermittent fever, strangury and cardiac debility.
- The stem is astringent, cooling, fragrant, diuretic, anthelmintic and useful in vomiting, leprosy and skin diseases.
- The roots are bitter, cooling, emollient, diuretic, and useful in pharyngopathy, pectoralgia, spermatorrhea, smallpox, diarrhoea, dysentery, cough, vitiated conditions of pitta.
- The leaves are bitter, cooling, diuretic, and are useful in burning sensation, hyperdipsia, fever, strangury, haemorrhoids and leprosy.
- The flowers are sweet, astringent, refrigerant and cardiac tonic. They are useful in diarrhoea, cholera, fever, hepatopathy, hyperdipsia, internal injuries, bronchitis, cough, skin eruptions and vitiated conditions of pitta.

- ❖ The stamens are cooling, astringent, diuretic, aphrodisiac and are useful in diarrhoea, hyperdipsia, haemorrhoids, inflammations, stomatitis and menorrhagia.
- ❖ The fruits and seeds are bitter, sweet, cooling, diuretic, tonic, depurative and aphrodisiac. They are useful in hyperdipsia dermopathy, halitosis, burning sensation, vomiting, menorrhagia, leucorrhoea, fever, pectoral diseases, leprosy and pruritis.

Nymphaea nouchali[14]

Taxonomical classification:

Kingdom **:** Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order **:** Nymphaeles

Family **:** Nymphaeaceae

Genus **:** *Nymphaea*

Species **:** *nouchali*

Nymphaea nouchali

Distribution:

Throughout the warmer parts of India, in tanks, ponds and ditches

Description:

A large perennial aquatic herb with short, erect, roundish, tuberous rhizome; leaves floating, peltate, sharply sinuate-toothed, flowers large, floating, solitary, variable in colour from pure white to deep red; fruits spongy many seeded berries, seeds minute, greyish black when dry with longitudinal striations

Part used: Rhizomes, Flowers, Seeds

Chemical constituents:

Apigenin, polysaccharides contain units of L-arabinose, D-xylose and small amounts of D-galactose and D-mannose.

Properties and uses:

- The rhizome is cooling, sweet, bitter and tonic, and is useful in diarrhoea, dysentery, dipsia and general debility.
- The flowers are astringent and cardio tonic.
- The seeds are sweet, cooling, constipating, aphrodisiac, stomachic and restorative.
- They are useful in vitiated conditions of *pitta,* dipsia, diarrhoea and dermatopathy.

Cyperus rotundus

Taxonomical classification

Kingdom **:** Plantae

Division : Mangnoliphyta

Class : Liliopsida

Order **:** Poales

Family **:** Cyperaceae

Genus **:** Cyperus

Species **:** rotundus

Cyperus rotundus

Distribution:

Throughout India, as a weed in waste lands from sea level to 1800m.

Description:

A perennial glabrous herb with elongate slender stolons bearing hard black fragrant tubers and triquetrous aerial stems; leaves numerous, narrowly linear, finely acuminate, flat, one nerved; spikelets in compound expanded umbels, spikelets linear to lanceolate, glumes imbricate; nut trigonus, broadly obovoid, greyish black

Part used: Tubers

Chemical constituents:

Cyperone, myrtenol, caryophyllene oxide, β-pinene, a-pinene, myrtenol, a-seliene[15] Isorotundene, cypera-2,4 (15)-diene, norrotundene and ketone-cyperadione, rotundene[16] carbohydrates, saponin, flavonoid, alkaloid, β cyanins, quinones, terpenoids, phenols, coumarins, proteins, steroids

Properties and Uses:

- The tubers are bitter, acrid, astringent, cooling, anti-inflammatory, revulsive, galactagogue, depurative, intellect promoting, nervine tonic, digestive, carminative, anthelmintic, stomachic, constipating, diuretic, lithotriptic, expectorant, diaphoretic, Emmenagogue, vulnerary, febrifuge, antiperiodic and tonic.
- Useful in vitiated conditions of *Kapha* and *Pitta*, hyperdipsia, inflammations, agalactia, leprosy, skin diseases, scabies, erysipcias, pruritis, amentia, neurasthenia, epilepsy, anorexia, dyspepsia, flatulence, colic, verminosis, diarrhoea, dysentery, strangury, renal and vescical calculi, cough bronchitis, amenorrhoea, dysmenorrhoea, wounds, ulcers, fever intermittent and malarial fevers, vomiting, ophthalmia and general debility.
- It is used to treat fever, digestive disorder, dysmenorrhoea and other malaties.
- It is used to treat skin disease like eczema, scabies and itching.
- Helps to burn fat deposition in obese people.
- It is a nerve relaxant and helps to treat diseases like psychosis, epilepsy and mental health problems[17]

Glycyrrhiza glabra[18]

Taxonomical classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Fabales

Family : Fabaceae

Genus : *Glycyrrhiza*

Species : *glabra*

Glycyrrhiza glabra

Distribution:

Cultivated in Punjab and the sub Himalayas tract.

Description:

A tall perennial under shrub about 1m high, leaves compound, leaflet 4-7 pairs; flowers violet in racemes; pods, oblong to linear, flattened, seeds reniform. The liquorice of commerce in the dried underground stems and roots.

Its outer surface is pale, chocolate brown in colour, flexible, fibrous and internally has a light yellow colour. It has a characteristic pleasant sweet taste.

Part used: Roots.

Chemical constituents:

Glycyrrhizin, glycyrrhetic acid, glycyrrhetinic acid, 24-hydroxy glycyrrhetic acid, mixture of potassium and calcium salts of glycyrrhizinic (glycyrrhizic) acid, glabrin A and B, glycyrrhetol, glabrolide, isoglabrolide, formononetin, glabrone, neoliquiritin, hispaglabridin A and B; heriniarin, umbellifrone; licoagrodin, glabrol, onocerin, β-amyrin, stigmasterol, β-sitosterol, glabroisoflavanone A and B, glabrocoumarin, glychionide A and B and flavanoides.

Properties and uses:

- The roots are sweet; refrigerant, emetic, tonic, diuretic, demulcent, mild laxative, aphrodisiac, expectorant, emmanagogue, alexipharmic, and intellect promoting.
- They are useful in hyperdipsia, cough, bronchitis, vitiated conditions of vatha, cephalalgia, fever, skin diseases and opthalmopathy.
- An extract of the root is good for treating gastric ulcers. A decoction of the root is a good wash for falling and graying of hair. Externally the root is applied for cuts and wounds.

Syzygium aromaticum[19]

Taxonomical classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Myrtales

Family : Myrtaceae

Genus : *Syzygium*

Species : *aromaticum*

Syzygium aromaticum

Distribution:

Cultivated mainly in Tamil Nadu and Kerala

Description:

An evergreen tree, 9-12m high or more. Leaves ovate-oblong, acute at both ends, gland-dotted, fragrant. Flower-buds borne in terminal, small clusters of branches, greenish; pink at maturity, aromatic. Fruits fleshy, dark pink dupes. Seeds oblong, grooved in one side.

Part used: Dried flower-buds

Chemical constituents:

Mainly β-caryophyllene; eugenol and its acetate, methylsalicylate; η-amylcarbinol, benzyl alcohol, dimethylfurfural, furfuryl alcohol, α-methylfurfural, methyl alcohol, methylvenzoate, methylfurfuryl alcohol nethyl-n-hepatylketone, methyl-n-hepatylcarbinol, valeraldehyde, vanillin, 2,6-dimethyl-5-hydeozy-7-methoxychromone (Eugenitine), 2,8-dimethyl-5,7-dihydroxychromone (Isoeugenitol), 2-methyl-5-hydroxy-7-methoxychromone (Eugenine), 2,4,6-trimethoxybenzoyl acetone (Eugenone).

From flowers-bud oil of wild cloves; furfual, methyl alcohol, naphthalene (Clove stem oil); benzaldehye, carvacrol, α-humulene, methyeugenol, eugenine, eugenone, epoxydihydrocaryophylle, methyl-n-amylketone, methyllpentanone, naphthalene (Leaf oil); caryophylla-3(12), 6-dien-4-ol, caryophylla-3(12), 7(13)-dien-6 α-al, caryophyllene oxide, 2-hydroxy-4,6-dimethoxy-5-methylaceto-phenone (Clove oil); galactose, glucose, fructose, rhamnose, csucrose, xylose, gallotannic and oleanolic acids are isolated from the cloves

Properties and uses:

- **Dried flower-buds:** Antispasmodic, aromatic, carminative, stimulant
- Beneficial in colic, dyspepsia, flatulence and various forms of gastric irritabsility, sore throat and in strengthening of gum, infusion given to allay thirst.
- Paste applied externally with much benefit in coryza and headache.
- Employed in Ayurvedic formulations for bronchitis, debility, dyspepsia and giddiness.
- Clove oil is antiseptic, local anesthetic, counter irritant and rubefacient.
- Externally applied in lumbago, neuralgia, rheumatic pains, sciatica and tooth ache.

Ziziphus mauritiania[20]

Taxonomical classification

Kingdom : Plantae

Division : Tracheophyta

Class : Magnoliopsida

Order : Rosales

Family : Rhamnaceae

Genus : *Ziziphus*

Species : *mauritiana*

Ziziphus marutiana

Distribution:

Found wild throughout India in waste places or tropical forests and in the outer Himalaya upto 1500m. also occurs in Sri Lanka, Malacca, Afghanistan, China, Australia, Tropical Africa and Burma

Description:

A large thorny shrub or small tree with rough grey or black bark; prickles on stems, young branches softly pubescent. Leaves simple, alternate, 2-6 cm long, variable, oblong-elliptic, ovate or subobicular, serrate or entire, three nerved, glabrous above covered beneath with a dense whitish or buff tomentum, prickles solitary or in pairs, 2.5 cm long. Flowers bisexual, greenish-yellow in small axillary clusters or short peduncled axillary cymes. Drupes globose or ovoid, succulent, fleshy, smooth, yellow or orange when ripe, stone 1-2 celled. Flowering and Fruiting: September--January

Part used: Fruit, Stem bark, Leaf, Root, Seed

Chemical constituents:

Plant: Jujuboside D, jujuboside A, 5,7,4'-trihydoxyflavonol-3O-beta-D-rhamnopyranosyl-(1->6)-beta-D-glucopyranoside, phenylalanine, jujuboside E, jujuboside B, jujuboside A, betulic acid, surose, inosine.

Leaves: Flavanoids, ziziphin, 13C-frangulamine, yuziphine, yuzirin as ®-1-(4'-hydroxybenzyl)-7-methoxy-8-hydroxy tetrahydrosioguinoline and 1(4'-hydrozybenzyl)-6-methoxy-7-hydroxyisoghinoline, cocklaurine, isoboldine, norisoboldine, asimilobine, n-octacosanol, alphitolic acid and saponin composed of abetin lactone, glucose, arabinose, 6 deoxy-L-mlose, ceanothic acid, betulinic acid, rutin.

Fruit: sapogenin-zizogenin, dammarane sapinin I, II and III, jujuboside B, flovone-C-glucosides-6"-sinapoylspinosin, 6"-feruloylspinosin and 6"-p-coumaroylspinosin, colubrinic acid, alphitolic acid, 3-O-cis-p-coumaroyl-maslinic acid, 3-O-trans-p-coumaroylmaslinic acid, betulinic acid, oleanolic acid, betulonic acid, zizyberenalic acid, fatty acids, carotenes, frangufoline, a flavonoid-spinosin, carbohydrates, fat, protein, amino acids, anthocyanins, leucoanthocyanins, catechins, cytokinin like zeatin, cyclic guanosine 3',5' monophosphate, carotene, citric, folic and malic acids, oleic acid, alphitolic acid, palmitoleic, vaccinic acid, zizyphus-pectin A, reducing and non-reducing sugars, niacin, riboflavin, thiamine, vitamin C, vitamin B, quercetin, cyclic Amp, jujubosie A,B, berberine, protopine eriodictyol, myricctin 3-O-glucoside, 3-O-rutinoside, rhamnetin, lauric acid, myristic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid, linoleic acid, arachidic acid, docosanoic acid.

Stem bark: Leucocyanidin, leucopelargocyanidin, amphibine-H, jubanies A and B, mucronine D and A, hummularines A and B, sapogenins as hecogenin acid, cocogenin, chlorogenic acid, mucronine-D, maslinic, usolics, 2α hydroxyursolic acid, mauritinen A, B, C, D, E, F, G, jubanine-C, scutianine-C and zizyphine-A.

Seed: Jujuboside A and B, 1,3-di-O-[9(Z)-octadecenoyl]-2-O-[9(Z), 12(Z)-octadecadienoyl]glycerol and a fatty acid mixture of linoleic, oleic and stearic acids, 3-O-[9(Z)-octadecenoyl]betulinic acid, and betulinic acid, jujuboside A1 and C and acetyl jujuboside B, protojujubosides A, B, and B1.

Substitutes and Adulterants:

There are few varieties of jujube under cultivation and are used as substitute. Besides these, fruits of z.*oenoplia* Mill., *Z.xylopyra Wild.*, *Z.rugosa Lam.*, *Z.sativa Gae*, *Z.nummularia W and A* , are sometimes used as substitute or adulterants.

Properties and uses:

- The ripe fruit is indigestible, aphrodisiac, anodyne, astringent, cooling, stomachic, styptic, tonic, expectorant, mild laxative and removes impurities from the blood.
- Leaves and twigs paste applied to abscesses, boils and curbuncles to promote suppuration and to strangury.
- Stem bark astringent, powder or decoction useful in diarrhoea, dysentery and in boils.
- Root bark juice is purgative, externally applied to gout and rheumatism.
- Decoction of root is beneficial in fever and powder for old wounds and ulcers
- Seeds are acrid and sweetish, tonic, antidiarrheal.
- Kernel used for abdominal pain in pregnancy and an antidote to aconite poisoning.
- It is used as antiemetic, sedative, sodorific and also cures eye diseases.
- Leaves astringent and diaphoretic.

Dryobalanops aromatica[21]

Taxonomical classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Magnoliopsida

Order : Malvales

Family : Dipterocarpaceae

Genus : *Dryobalanops*

Species : *aromatica*

BORNEO CAMPHOR:

Distribution:

A genus of tall tree, Yielding camphoraceous-oleoresin, distributed in Sumatra from bornea.

Undisturbed mixed dipterocarp forests at elevations up to 300 metres. Usually found growing on hillsides and ridges with sandy soils. In secondary forests it is usually present as a pre-disturbance remnant tree.

Description:

Borneo Camphor is a tall, evergreen tree with a large, globose crown; it usually reaches a height of 40-50 metres, with occasional specimens up to 60 metres tall. It has a straight bole that can be un branched for 30 metres and is usually 1-1.5 metres in diameter with exceptional specimens to 3 metres.

The tree is a source of camphor, used medicinally and in perfumes, and is also the most important source of a high quality timber, known as kapur, which is used locally and also traded.

The plant has a long history of medicinal use, with evidence that it has been traded internationally since at least the 6^{th} century AD.

The tree is classified as critically endangered in the IUCN Red list of Threatened Species (2009), with only small subpopulations of the species having been found in forest reserves.

Properties:

Borneo camphor is found in cavities or fissures in the wood of *D. aromatica* and is collected by scraping.

It occurs in white translucent masses and closely resembles true camphor from *Cinnamomum camphora* in many aspects.

It is distinguished by not volatilizing at ordinary temperature and possessing a characteristic pungent odour, burning taste.

It is used in same manner as camphor in medicines and perfumery. It is also employed in organic synthesis Borneo camphor is highly prized in Indian medicine (Wealth of India).

Chemical constituents:

- Borneol
- Camphene
- Terpeniol
- Sesquiterpene

Chemically, Borneo camphor is almost pure d-borneol.it is converted into ordinary camphor by treatment with boiling nitric acid.

Oryza sativa [22]

Taxonomical classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Liliopdida

Order : Poales

Family : Poaceae

Genus : *Oryza*

Species : *sativa*

Oryza sativa

Distribution:

Throughout India, cultivated.

Description:

An annual or perennial grass with tuft of fibrous roots and swollen nodes; leaves simple with sheathing bases, long and narrow, slightly pubescent with spiny hairs on the margins; flowers spikelets in terminal compound panicles, lemma punctate of granulate without wing on the back, lemma and palea surrounding the

kernel, golden yellow, reddish purple, brown or smoky black becoming straw-colored on ripening, grains narrowly oblong, free within the lemma and palea.

Part used: Roots, Grains

Chemical constituents:

Carlinoside, flavonoid pigments, glucotricin, oryzalexin A, B and C, alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, triaceylglycerols, acylated steryl glucoside, phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, stachydrine, trigonelline, stigmastanol, starch, glucose, detrin, fructose, galactose, raffinose, maltose, arabinose and xylose, mannose, uronic acid, a glutelin as oryzenin, albumin, α and β globulins and prolamines, tryptophan, phenylalanine, xanthine, adenine, hypoxanthine, ammonia, vitamins E and B, pantothenic acid, pyridoxine, enzymes like α amylase, β amylase, amylosynhease, lipase, phenolase.

Properties and uses:

- The roots are cooling, diuretic and febrifuge. Useful in burning sensation, dispepsia, bilious fever, strangury and diabetes.
- The grains are sweet, acrid, oleaginous, aphrodisiac, diuretic, carminative, antidysenteric and tonic.
- They are useful in vitiated conditions of *Pitta*, pneumonosis especially pulmonary consumption, diarrhoea and clolnopathy.
- Seeds with milk beneficial in peptic ulcer, powder dusted over surface has a cooling and soothing effect on burns and scald.
- It is beneficial in erysipelas, measles, pox, prickly heat and other inflammatory affections of the skin.
- As poultice applied to abscess, boils, buboes, inflammatory affections, piles and ulcers also to chest in chronic bronchitis and cough.
- Rice-water used in febrile and intestinal disorders as demulcent, refrigerant and soothing.
- Rice gruel useful in impaired digestion like diarrhoea and dysentery.

3.3 LITERATURE REVIEW OF DISEASE

3.3.1 SIDDHA ASPECT OF THE DISEASE

KURUTHI AZHAL NOI:

Definition:

It refers to the functional divergence in seneer thathu (blood), produced as a results of increased azhal (bio energy fire) eventully leading to consequent functional dearrangements in other udal thathus.

Vernacular names:

"இரத்த வமுக்கத் தியர்பெய ருரைத்திடிற்

இரத்தாதிக்கம் யழுத்தம் இரத்தக் கொதிப்பு

நந்திடும் நாடிஇறுக்கமும் நவில்வர்

பத்திடுங் காரணம் பகரக்கேண்மோ"

-நோய்நாடல் நோய்முதல் நாடல்திரட்டு[23]

Raktha soodu, Raktha azhutham, Raktha miguthi, Raktha perukkam, Rakthathikkam, Narambirukkam and Naadi irukkam.

Types of Disease:

The disease may be classified into eight types as follows:

1. Vali kuruthiazhal noi
2. Thee kuruthiazhal noi
3. Iya kuruthiazhal noi
4. Vali thee kuruthiazhal noi
5. Vali iya kuruthiazhal noi
6. Iya vali kuruthiazhal noi
7. Iya azhal kuruthiazhal noi
8. Mukkutra kuruthiazhal noi

Some other groups of ancient physicians have classified this disease into four types depending upon the direction of flow of blood as follows:

1. Maelnokku kuruthiazhal noi
2. Keelnokku kuruthiazhal noi
3. Irunokku kuruthiazhal noi
4. Alavilla nokku kuruthiazhal noi[24]

Clinical features:

- ❖ Head ache most commonly seen in occipital region, dizziness, palpitation, easy fatigability, weakness.
- ❖ Dysponea, chest pain, pallor, perspiration, vertigo, syncope.
- ❖ Epistaxis, heamaturia, blurring of vision.

Epidemiology:

"கேட்டிடு பன்னாட்கெடு மலச்சிக்கல்

கூட்டிடும்மதுக் கள்கொள்ளல் புகையிலை

நாட்டிடும் மாவகை நற்கொழுப்பிறைச்சி

ஊட்டுதல் மிகுதல் ஊழைப்புமிகுதி

மிகுமனக்களிப்பு மிகுநாடித்துடிப்பு

தரு மனவோட்டம் நாடுதல்காமம்

தருமேகநோயிகள் தக்க பல்தொண்டை

பகு பித்தப்பை பலஉறுப்பினிலும்"

-நோய்நாடல் நோய்முதல் நாடல்திரட்டு

Kuruthi azhal noi is high in urban population and more common in men than women. In females the prevalence is closely related to age. This is increased presumeably related to the menopause.

- ❖ The elavenil (early summer), muthuvenil (late summer) aggressive the disease.
- ❖ Paalai (arid-tract) and neithal (coastal-tract) reported compared to other areas[23a]

3.3.2 MODERN ASPECT OF THE DISEASE

HYPERTENSION

The history of hypertension starts at 1628 when the book De Motu Cadrdis was published, and the book was written by an English physician Willium Harvey, who described the circulation of blood. Then the elergyman Stephen hales made the first measurement of blood pressure in 1733. Hypertension was described as a disease by thomus young and Richard in 1836. In 1884 the first report of elevated blood pressure in a person without evidence of kidney disease was recorded. Eventually in 1896 the invention cuff-based sphygmomanometer done by Scipione Riva-Rocci. Then the blood pressure was measured in the clinics, Nikolai korotkoff improved this technique by describing the korkoff sounds. These sounds are heard when the artery was ascultated with a stethoscope while the sphygmomanometer cuff was deflated.

Now in modern medicine, hypertension is classified into two type's essential and secondary hypertension. The concept of essential hypertension was introduced in 1925 by the physiologist Otto frank, which has idiopathic origin. In 1928 the term malignant hypertension was coined by physicians from the mayo clinic. The essential hypertension may be the result of a combination of poor life style choices and genetics. Secondary hypertension that starts as a result of another disease especially kidney disease or disease associated with endocrine system [25]

Definition:

Systemic hypertension is the persistent rise of basal blood pressure above the level of 140/90mmHg recorded on three or more successive occasions [26].

Classification of hypertension

- Essential or idiopathic hypertension
- Secondary or symptomatic hypertension

Table 3: Classification of blood pressure for adult (JNC7) [27]

Category	**Systolic mmHg**	**Diastolic mmHg**
Normal	90-119	60-79
High normal (pre-hypertension)	120-139	80-89
Stage I Hypertension	140-159	90-99
Stage II (Hypertension)	160-179	100-109
Stage III (Hypertensive emergency)	≥ 180	≥ 110
Isolated Hypertension	≥ 140	< 90

White coat Hypertension

It is commonly known as white coat syndrome or masked hypertension, is a phenomenon in which patient exhibit a blood pressure level above normal range, In a clinical setting, though they don't having exhibit other it in other settings. It is believed that the phenomenon is due to anxiety that those afflicted experience during a clinical visit [28].

Pathophysiology of Hypertension

Blood pressure is determined by the balance between cardiac output and vascular resistance rise in either of these variables, in the absence of a compensatory decrease in the other, increases mean Bp, which is the driving pressure.

Factors that affect cardiac output include the following:

- ❖ Baroreceptors
- ❖ Extracellular volume
- ❖ Effective circulating volume, Arterial natriuretic hormones, Mineralocorticoids, Angiotensin
- ❖ Sympathetic nervous syndrome

Factors that affect vascular resistance include the following:

- ❖ Depressors-angiotensin II, calcium (intracellular), catecholamines, vasopressin.
- ❖ Depressors-Artial natri uretic hormones, endothelial relaxing factors, kinins, prostaglandin, prostaglandin E_2, prostaglandin I_2

Changes in the electrolyte homeostasis particularly in sodium, calcium and potassium concentrations, affect some of these factors.

Under normal conditions, the amount of sodium excreted in the urine matches the amount of ingested, resulting in near constancy of extracellular volume. Retention of sodium results in increased extracellular volume, which associated with elevation of Bp. By means of various physical and hormonal mechanisms, elevation triggers changes in both the glomerular filtration rate (GFR) and the tubular reabsorption of sodium, resulting in excretion of excess sodium and restoration of sodium balance.

A rise in the intracellular calcium concentration, due to changes in plasma calcium concentration, increases vascular contractility. In addition, calcium stimulates release of renin, synthesis of epinephrine and sympathetic nervous system activity. Increased potassium intake suppresses the production and release of renin and induces natriuresis, decreasing the Bp.

Table 4: Common causes of hypertension by age[29]

Infants	Children 1-6 yrs	Children 7-12
Thrombosis of renal artery or vein	Renal artery stenosis	Renal parenchymal disease
Congenital renal anomalies	Renal parenchymal disease	Renovascular abnormalities
Coarctation of aorta	Wilms tumor Neuroblastoma	Endocrine causes
Bronchopulmonary dysplasia	Coarctation of aorta	Essential hypertension

Causes of primary hypertension:

Also commonly known essential hypertension, is a disorder which is associated with elevated blood pressure whose causes are not readily identifiable. Its prevalence tends to rise with age in most populations. Essential hypertension is common in adults.

The following risk factors for primary hypertension:

- Obesity or being overweight
- Excessive salt consumption
- Family history and genetics
- Sedentary life style[30]

Causes for secondary hypertension

- Renal causes- acute nephritic syndrome, chronic nephritis, Poly cystic kidney, Hydronephrosis, chronic pyelonephritis, Renal artery stenosis, Renin secreting tumour, Renal embolism.
- Endocrine causes- thyrotoxicosis and myxoedema, acromegaly, cushing's syndrome, cohn's syndrome, pheochromocytoma.
- Metabolic causes- diabetes mellitus, chronic gout, toxemias of pregnancy, atherosclerosis.
- Drugs- contraceptive pills, steroids, liquorice.

- Collagenosis and miscellaneous disease- SLE, polyarteritis nodosa, scleroderma, dermatomyositis, pseudoxanthoma elasticum.
- Congenital- coarctation of aorta.
- Psychogenic
- Neurological- encephalitis, brain tumour, cerebro vascular accident, diencephalic Syndrome.
- Blood disease- Polycythemia
- Renovascular hypertension- particularly in renal artery stenosis
- Miscellaneous- Pregnancy, Cyclosporine, NSAIDs.[30]

Clinical features:

- Pulsating head ache often in occipital and occurs particularly in the morning.
- Easy fatiguability
- Insomnia
- Dizziness
- Lack of concentration
- Loss of memory
- Occational palpitation

Symptoms of associated disease may also present e.g. Cerebral Arteriosclerosis, Retinal Arteriosclerosis, Coronary Arteriosclerosis, Renal arteriosclerosis and Arteriosclerosis of limb of vessels [31].

Complication:

Cardiac hypertensive heart disease

Left Ventricular Hypertrophy develops in 10-30% of chronic cases. It may produce myocardial ischemia, ventricular arrhythmia, CCF and sudden death; LV diastolic dysfunction may also develop with CCF.

Cerebral

Cerebro vascular complications are more closely to systolic rather than diastolic blood pressure.

- Cerebral hemorrhages
- Cerebral thrombosis

- Lacunars infarct
- Hypertensive encephalopathy- this condition is acute cerebral ischemia from Hypertensive spasm, Cerebral edema and minor degree of hemorrhages or thrombosis.
- TIA- transient ischemic attack
- Subarachnoid hemorrhages.
- Dementia- both vascular and Alzheimer's type

Retinal

- Dimness of vision
- Thickening of arteries with Narrowing of lumen, Hemorrhage.
- Papilloedema.
- Detachment of Retina, Vitreous Hemorrhages.

Renal

Patients with hypertensive nephropathy should have BP at 130/85mmHg or less if proteinuria is present. Hypertension accelerates all forms of renal disease mostly diabetic nephropathy.

- Nephrosclerosis
- Uremia
- Renal infarct

Aortic dissection

- The major cause of hypertension.

Atherosclerotic complications

Many patients of hypertension die out of these complication but the relationship is much less close than other complications.

- Cerebral arteriosclerosis
- Retinal arteriosclerosis
- Coronary arteriosclerosis
- Renal arteriosclerosis
- Arteriosclerosis of limb vessels.

Pregnancy induced hypertension

Gestational hypertension also referred to pregnancy induced hypertension (PIH). It is a condition of high blood pressure more than 140/90mmHg on two separate occasions, more than 6 hours apart, without the presence of protein in the urine during pregnancy. It is diagnosed after 20 weeks of gestation. Gestational hypertension can lead to serious condition called pre-eclampsia[32].

Hypertensive crisis

In some situations in hypertensive patients rapid reduction of blood pressure is required. These situations are including under the category of hypertensive crisis. These situations divided into two.

1. Hypertensive urgencies

Where blood pressure reduction is required comparatively slowly, Diastolic pressure is more than 130mmHg.

2. Hypertensive emergencies (Malignant hypertension)

Where immediate (within one hour) reduction of blood pressure is required. Systolic blood pressure is greater than 210mmHg of diastolic blood pressure 130mmHg.

Clinical features include

- Headache
- Confusion
- Visual loss
- Focal Neurologic features
- Somnolence
- Coma
- Fundus shows Hemorrhages, Exudates and Papilloedema.

Mode of termination

- Acute left ventricular failure (60%)
- Cerebral hemorrhages (35%)
- Uremia rare(5%)[33]

Differential diagnosis:

Aortic coarctation:

Differential blood pressure in upper and lower extremities. Absent of femoral pulses.

Obstructive sleep apnea:

Typically obese patients with day time somnolence, snoring, or choking during sleep [34].

Investigations:

- Urine analysis, Protein and Glucose
- Blood, Urea, Electrolytes and creatinine
- Blood glucose
- Serum total and high-density lipoprotein(HDL) Cholesterol
- 12-lead ECG (left ventricular Hypertrophy, Coronary artery disease)
- Endocrine: Serum Sodium, Potassium, Calcium and TSH[33a]

3.4 TREATMENT FOR HYPERTENSION

3.4.1 *SIDDHA* ASPECT

In siddha system the treatment for *Kuruthi azhal noi* is based on the normalizing the altered hypertension.

Vanthi* and *kazhichal maruthuvam

(Bowel cleansing method for *azhal* and *vayu thathu*)

- *Meganatha thylam*-8-30ml at early morning
- *Vellai ennai*-15-30 ml at early morning
- *Karuda kizhangu thylam*-15 ml at early morning
- *Sanjeevi mathirai*-1-2 at early morning with goat's milk(50ml) kudineer
- *Kowshikar kuzhambu*-125-500 mg with ghee at early morning
- *Thiratchai kudineer*-40-80ml twice a day

The choices of medicine, doses and duration may be altered according to the condition of the patients and severity of the disease.

Chooranam

- *Marutham pattai chooranam*-1-2 g twice a day with hotwater(50ml)
- *Ceeraga chooranam*-1-2 g twice a day with hotwater(50ml)
- *Thirachathy chooranam*-1-2g twice a day with honey(5ml)
- *Seenthil chooranam*-1-2g twice a day with ghee(5ml)
- *Thirippala chooranam*-1-2g twice a day with hotwater(50ml)
- *Thalisathy chooranam*-1-2g twice a day with honey (5ml)
- *Elathy chooranam* 1-2g twice a day with warm water(50ml)
- *Amukkara chooranam*1-2g twice a day with milk(50ml)

Nei

- *Brahmi nei* 10-15ml twice a day with milk(50ml)

Manappagu

- *Thurunchi manappagu* 15ml twice a day with luke warm water (50ml)
- *Nannari manappagu* 15ml twice a day with luke warm water (50ml)
- *Madhulai manappagu* 10-15 ml twice a day with luke warm water(50ml)

Ilagam

- *Thetran ilagam* *5*-10g twice a day
- *Vilvathy ilagam* 5-10g twice a day
- *Madhulai manappagu* 3g twice a day

Karpam

- *Bavana kadukkai* (500mg) 1 before and after food twice day

Parpam

- *Silasathu parpam* 300-600mgtwice a day with hotwater(50ml)
- *Kungiliya parpam* 100-300mg twice a day tendercoconut water(50ml)
- *Sangu parpam* 100-300mg twice a day with ghee(5ml)
- *Nathai parpam* 65-130mg twice a day with ghee(5ml)
- *Siringi parpam* 65-130mgtwice a day with brahmi nei(10ml)

Chenduram

- *Vediannabethi chenduram* 100-200mg twice a day with honey(5ml)
- *Aya chenduram* 65-130mg twice a day with honey(5ml)
- *Ayakandha chenduram* 65-130mg twice a day with honey(5ml)

Karpa marunthugal

Pothu karpam

- *Kattrazhai karpam* for 4 days
- *Ponnangani karpam* for 4 days
- *Kaiyan karpam* 1-2gms for 2 months

Sirappu karpam

- *Kadukkai karpam 1-2* gms with hotwater at evening for 48 days
- *Panai ver kudinner* 60ml twice a day for 48 days
- *Vilva karpam* for 48 days
- *Elumitchai pazha karpam* for 48 days
- *Orilai thamarai karpam* with ghee for 48 days.

External medicines

Oil bath may be advised twice a week with any of the following medicated oil.

- *Kaiyan thylam*
- *Ceeraga thylam*
- *Keezhaneli thylam*
- *Lahusandhanathy thylam*
- *Arakku thylam*
- *Thirippala thylam*

Pathiyam(diet)

Diet to be added

- Rice kanji-double boiled rice, *savvarisi kanji, pori kanji, barley kanji, manakkathai, kuruvai rice.*
- Vegetables- *athi, avarai, kathari, vazhai, vendai, murungai, sundai, mullangi, pagal, sambal poosani, thoothuvalai, pirandai.*
- Greens- *puliyarai, manathakkali, ponnangani, kaiyan, sukkan, vasalai keerai, pasalai keerai.*
- Pulses- *ulunthu, pasipayaru*
- Dairy products- cow's butter milk
- Non-vegetarian diet- *ayrai meen* (loach), *velladu* (capea hircus)

Diet to be avoided

- Intake of excessive salt, oil, fried food.
- Excessive hot, sour and salt tastes.
- *Sarkkarai valli kizhangu* (Ipomoea batatas), *Seppankizhangu* (Colacasiaesculanta), *Kothavarai* (Cyamopsis tetragonoloba), *Kollu* (Macrotylorum uvifiorum), *Verkadalai* (Arachis hypogea), *Kaaramani* (Vigna ungiculata), *Pattani* (Pisum sativum), *Motchai* (lablab purpureus).

Other instructions

- Avoid smoking and alcohol intake
- Dietary management
- Regular physical activity (brisk walking) at least 30 min/day
- Weight reduction, maintain normal body weight
- Relief of stress.
- Rejuvenation therapy

Karpayogam

- *Pranayamam*
- *Singasanam*
- *Sarvangasanam*
- *Puyangasanam*

3.4.2TREATMENT FOR HYPERTENSION IN MODERN ASPECT

Table 5: Classification of Anti-Hypertensive Drugs

S.NO	TYPES OF DRUG	NAME OF THE DRUG
1.	ACE inhibitors	Captopril Enalapril Lisinopril Ramipril
2.	Angiotensin antagonist	Losartan

3.	Calcium channel blockers	Nifidepine Felodipine Amlodipine Verapamil Diltiazem
4.	Diuretics	Hydrochlorothiazide Fruosemide Indapamide Spironolactone Triamterene Amiloride
5.	β-adrenergic blockers	Propranolol Atenolol Metaprolol
6.	α-adrenergic blockers	Prazocin Terazocin Phentolamine
7.	Central sympatholytics	Clonidine Methyldopa
8.	Vasodilators (i)Arteriolar (ii)Arteriolar and venular	Hydralazine Minoxidil Diazoxide Sodium nitroprusside Pinacidil

Anti-hypertensive drugs act by influencing the Blood pressure regulatory systems viz, the autonomic nervous system, Renin Angoitensin System. Calcium channels or sodium and water balance in plasma volume [35].

Classification of anti-hypertensive drugs

Diuretics

Diuretics lower the blood pressure by increasing urination. The anti-hypertensive action of diuretics is mild BP falls by 15-20mmHg over 2-4 weeks.

Diuretics enhance the excretion of sodium and water resulting in decreased plasma volume of CO and reduce BP.

- Thiazides (Hydrochlorothiazide, Chlorothiazide, Indapamide)
- Loop diuretics (Fruosemide, Bumetanide, Torsemide)
- K^+ (Spiranolactone, Amiloride, Trimterene)

Drugs acts on Rennin Angiotensin System

- Angiotensin converting enzyme inhibitors (ACE inhibitors) (Captopril, Enalapril, Lisinopril, Ramipril, Perindopril)
- Angiotensin II receptor antagonists (Losartan, Candesartan, Valsartan, Eprosartan, Irbesartan, Olmesartan)
- Rennin inhibitor(Aliskien)
- ACE inhibitors are presently the first line anti-hypertensive. ACE inhibitors are useful in the treatment hypertension of all grades due to all causes. They are specially indicated as Anti-hypertensives in hypertension with left ventricular hypertrophy.
- Angiotensin II is powerful vasoconstrictor. ARBs are used in the treatment of hypertension in similar indications as that of ACE inhibitors as alternatives of ACE inhibitors, they can also be considered as the first line drug in hypertension.
- Rennin inhibitors blocks the effects of rennin there by reducing blood pressure. Use of several drugs like ACE inhibitors, ARBs and diuretics tend to bring about a compensatory rise in the plasma rennin levels. Because aliskiren blocks the effects of rennin, its action is synergistic with these drugs.

Sympatholytics

- Centrally acting drugs (Clodine, Methyldopa, Guanfacine)
- Ganglion blockers (Trimethaphan)
- Adrenergic receptor blockers (Guanethidine, Reserpine)
- Adrenergic receptor blockers
- α-blockers (Prazosin, Terazosin, Doxazosin, Phenoxybenzamine, Phentolamine)
- β-blockers (Propranolol, Atenolol, metoprolol)
- α and β blockers (Labetalol, Carvedilol).

Sympatholytics drugs may be used to interfere with sympathetic activity at different levels including centrally, at the ganglia, neurons and receptors.

Ganglion blockers block both sympathetic and parasympathetic ganglia resulting in decreased sympathetic tone and a fall in BP.

Adrenergic receptor blockers depletes the stores of nor adrenaline in the adrenergic neurons blockers its release.

α-blockers are used in the treatment of hypertension due to Pheochromacytoma. They block the α1 receptors in the arterioles and venules and thereby dilate both arteriole and venules. Peripheral vascular resistance is decreased leading to fall in BP with only mild tachycardia. β-blockers are mild anti-hypertensives. Blockage of β1 receptors results in decreased myocardial contractility and cardiac output. Thus they reduce BP due to a fall in the cardiac output [36].

Ca+ channel blockers

CCBs are another important group of anti-hypertensive. They are particularly used in elderly patients. CCBs may be used as mono therapy or in moderate to severe Hypertension along with other hypertensives (Nifedipine, Nicardipine, Nimodipine, Amlodipine, Felodipine, Verpamil).

Vasodilators

- Arteriolar dilators (Hydralazine, Minoxidil, Diazoxide)
- Arteriolar and venular dilators (sodium nitroprusside)

Vasodilators relax the vascular smooth muscle thus reducing BP due to decresed peripheral vascular resistance. Nitroprusside is the drug of choice in hypertensive emergencies. It is used in situations where short-term reduction of myocardial work load is required as serve heart failure and myocardial infarction.

Adverse effects of anti-hypertensive drugs

- Angiotensin receptor blockers cause Hypotensin and Hyperkalaemia.
- Sympatholytics like Methyldopa cause dryness of mouth and nose, depression, vertigo, extra-pyramidal signs, raised prolactin levels, postural hypertension.
- Vasodilators like Hydralazine cause headache, dizziness, flushing, palpitation, nausea, anorexia, hypotension and salt and water retension.
- Sodium nitroprusside cause palpitation, sweating, weakness, nausea, vomiting and hypotension [37].

Future vaccine:

DNA vaccine

DNA vaccine that targets angiotensin II- a hormone that raises blood pressure by causing blood vessels to constrict. The narrowing can increases blood pressure and force heart to work harder.

In the study, researchers immunized hypertensive rats three times at two-week intervals with needless injections. The vaccine not only lowered blood pressure for up to six months, but also reduced tissue damage to the heart and blood vessels associated with hypertension. There were no signs of damage to other organs such as the kidney or liver.

The DNA vaccine works similar to common ACE inhibitor blood pressure medications which help blood vessels relax and open up, which in turn lowers blood pressure.

Other type of vaccine: Peptide vaccine [38].

3.5 PHARMACOLOGICAL REVIEW

PHARMACOLOGY STUDY OF ANTIHYPERTENSIVE ACTIVITY IN ANIMAL MODELS

HYPERTENSION MODELS

IN-VITRO MODEL

- Endothelin receptor antagonism in porcine isolated hearts
- Monocrotaline induced pulmonary hypertension

Endothelin Receptor model

Endothelins (ET) have been implicated in the pathophysiology of cardiovascular diseases. In this model isolated porcine coronary artery is used since the smooth musculature of artery is considered to contain the ETA receptors. ET results in potent long lasting contractions of isolated blood vessel strips. An increase of blood pressure in vitro studies has been elicited by Endothelin peptides.

Monocrotaline-induced pulmonary hypertension

- Monocrotaline is a hepatotoxic pneumotoxic agent used in Rats for pulmonary hypertension. It is a pyrolizidine alkaloid derived from Crotalaria spectabilis and single injection leads to progressive pulmonary hypertension followed by right ventricular hypertrophy and cardiac failure. Ultrastructural changes such as degeneration and fragmentation of endothelial cells and mascularization of pulmonary arteries, arterioles are also observed. Monocrotaline administration of rats can results in severe right ventricular hypertrophy accompanied by rats and pleural effusion.

IN-VIVO MODELS

RAT MODELS

I. Reno-vascular induced

- Two-kidney one clip method (Goldblatt hypertension, 2K1C)
- Chronic renal hypertension in rats (1-kidney-1-clip method)
- Chronic renal hypertension in rats (Two kidney two clip method)

II. Neurogenic induced

- Blood pressure in pithed rats

III. Diet induced

- Fructose induced
- Increased salt induced

IV. Endocrine induced

- DOCA-salt rats

V. Psychogenic

- Air-jet stimulation induced hypertension

VI. Genetically induced

- Salt-sensitive Dahl rats
- Spontaneously hypertensive rats

DOG MODEL OF HYPERTENSION

- Chronic renal hypertension
- Neurogenic hypertension

MONKEY MODEL OF HYPERTENSION

- Renin inhibition in monkeys

TRANSGENIC MODEL OF HYPERTENSION

- Transgenic rats overexpressing the mouse Ren-2 gene [TGR(mRen 2)27]

Two kidney one clip (Gold blatt hypertension2k1c)

Sprague dawley rats used for this model

Ischemia of the kidney causes elevation of blood pressure by activation of rennin angiotensin system. In rats clamping the renal artery for 4 hours can activates peripheral RAAS and sympathetic nervous system and induce renal hypertension. After reopening the vessel, accumulated rennin is released into circulation leading to acute hypertension. Renin is secreted by the kidneys when sympathetic activity is increased. Renin converts Angiotensin to Angiotensin I, angiotensin II is a potent vasoconstrictor and increase blood pressure. Angiotensin II also causes release of aldosterone leading to salt and water retention result in increased blood volume and hypertension. This model is used to evaluate anti-hypertensive activities of drugs.

Chronic renal hypertension (one kidney one clip method)

The one kidney one clip method is the technique has been described for several animal species. The most effective modifications in rats in which one kidney is removed. Constriction of one kidney is done on one side and the contralateral kidney is removed. There is an increase in blood pressure within few hours. Since there is no other kidney, there is no blood pressure, diuresis, natriuresis, so there is rapid salt and water retention. Plasma renin activity is usually normal hypertension soon becames volume dependent.

Blood pressure in pithed rats

Male wistar rats (250-350 gms) are used in this model

The pithed rat model is divided for neurogenic reflex control that may modulate the primary drug effect. It is frequently used to evaluate drug action on the cardiovascular system.

Salt-sensitive Dahl rats

The kidneys have the ability to excrete easily the daily salt load without allowing a marked rise in extracellular volume. Chronic ingestion of excess salt produces hypertension in rats, which mimics human hypertension. The salt-sensitive dahl develop severe and fatal hypertension when feed high salt diets. This is the model of genetic hypertension, with the extra feature of Salt sensitive.

Fructose induced hypertension in rats

Feeding a high fructose diet induces hypertension and insulin resistance in Sprague dawley rats. Fructose feeding also causes insulin resistance, hyperinsulinemia and hypertriglyceridemia in normal rats. Fructose feeding induces hypertension in normal or high salt feed animals and it is associated with an increased expression of the Angiotensin II type I receptor in adipose tissue. AT 1 receptor play a role in the pathophysiology of metabolic and hemodynamic abnormalities induced b fructose feeding.

DOCA-salt rats

Mineralocorticoids induces hypertension by causing in increased plasma and exracellular volume. The administration of deoxycorticosterone acetate (DOCA) , a mineralocorticoid in combination with high salt diet and unilateral nephrectomy induces hypertension. There is increased DOCA induced reabsorption of salt and water leading to increased blood volume and hence increased blood pressure [39].

Spontaneously hypertensive rats

By breeding strain of spontaneous hypertensive wistar rats with a female having slightly raised blood pressure, Okamoto and Aoki obtained a strain of rats spontaneous hypertension, Spontaneous Hypertension was devopled by meticulous genetic in breeding that uniformly resulted in 100% of progeny having naturally occurring hypertension. Several researchers reported that the spontaneous hypertension is an excellent model of experimental hypertension as well as model for complication of hypertension [40].

Transgenic rats overexpressing the mouse Ren2 gene [TGR(mRen2)27]

The ability to specifically introduce genetic constructs and thereby breed transgenic animal has opened new possibilities for hypertension research.Recently a transgenic rat has been obtained after introduction of the entire mouse Ren 2d gene. The introduction and over expression of mouse Ren2 in this rat leads to severe, lethal in the homozygous rat.

Method for the measurement of blood pressure in rats

- Tail-cuff method in rats
- Indwelling catheter for measurement of blood pressure in conscious rats.

Tail cuff method for measuring Bp

The indirect tail cuff method allows the measurement of Bp without any surgical procedure. The principle used in this method is that the pulse obliterate when the cuff is inflated will above suspected systolic blood pressure. The pulse reappears as the pressure in the cuff is slowly released and it falls below the systolic blood pressure. The method is analogous to sphygmonometry in humans.

The indirect tail cuff method is widely used to evaluate the influence of anti-hypertensive drugs in spontaneously and experimentally induced hypertensive rats.

Indwelling catheter method

This method allows directly measurement of blood pressure in conscious rat. The influence of anesthesia on the cardiovascular regulation is eliminated by this method.

7 cm long cannulae are prepared by cutting pe 10 and pe 20 tubing respectively. A style wire is inserted into the pe10 tubing is also slipped over the style wire. The tube ends are heated in a current of hot air and fused together. Using ridges the style wire is left inside the cannula and the cannula is heated in a jet of hot air. When the polyethelene at the point of heating becomes soft, the cannula is pressed slightly and the ridges are formed.

Dog models of hypertension

- Chronic renal hypertension
- Neurogenic hypertension

Chronic renal hypertension

Partial constriction of renal arteries in dogs produces hypertension. This method is modified and is now known as the wrapping technique, a sheet of cellophane is placed around the kidney and held in place by silk sutures tied loosely around the renal hilus. Both kidneys are wrapped or one kidney is wrapped and other is removed. A fibro collagenous shell is formed around the kidney in 3-5 days because of reaction of the tissue to the foreign material. This shell compresses renal vascular pressure. This expands the extracellular volume leading to increased peripheral resistance and hence increased blood pressure.

Neurogenic hypertension

Baroreceptor situated in the carotid sinus and aortic arch play an important part in the regulation of blood pressure. Stimulation of baroreceptor causes inhibition of vasomotor center leading to vasodilatation, bradycardia and decrease in blood pressure. Sectioning of the baroreceptor nerves leads to persistant raise in blood pressure. Thus these produce acute neurogenic hypertension in dogs.

Renin inhibition method in monkey

Blood pressure is mainly regulated by the renin Angiotensin system and can be influenced by the inhibition of renin. Renin inhibitors developed for have a high specifically for primate rennin and cause only weak inhibition of renin sub primate species. It suggests that most commonly laboratory animals such as dog are not suitable for in-vivo evaluvation of rennin inhibitor. Marmosets (callithrix jacchus) of 300-400 gare feed pellet diet supplemented with fruits. These animals were used for rennin inhibition method [41].

ANIMAL MODEL FOR THE DISSERTATION

SPONTANEOUSLY HYPERTENSIVE RATS:

Systolic blood pressure (SBP) and heart rate measurement of SH rats was carried out using tail-cuff method plethysmography (LE 5001 Pressure Meter). A mean of six measurements was obtained for each animal. For blood pressure measurement, the animals were warmed up to 42°C for 5 min in a confinement cage. The animals were first submitted to a period of adaptation for 15 days before the experiments and only SHR with an SBP > 170mmHg were selected for this study.

During the final week of the treatment, the rats were allowed to acclimatize to the experimental conditions of non-invasive SBP measurements by allowing them to stand in rat restrainers for 30 min every day. SBP measurements were recorded 24 hours after the last treatment dose. At least 8-10 recordings were taken for each rat and the mean of the lowest 4 values within less than 10 mmHg difference was taken as the mean SBP.

3.6 PHARMACEUTICAL REVIEW

CHOORANAM

Definition:

Chooranam is a fine powder of drugs. The "Chooranam" may be applied to the powders of a single drug or a mixture of two or more drugs, which are powdered separately prior to their being mixed to homogeneity [42].

Method of preparation:

Equipment required

- The drug enumerated in the recipe in clean and well dried state.
- A mortar and pestle.
- A fine sieve or fine cloth of close mesh.

Process of preparation:

The drugs which are to be used in the preparations should be taken from recently collected material. Drugs which are aged by prolonged storages or changed in colour, taste and scent, and those that are insects infested or attacked by fungi should be positively rejected.

However drugs like Embelia fruits, Senna, Long Pepper, Jaggery and cows ghee are preferred from fairly aged stock, provided they are not infested with pests, deteriorated or spoiled or developed racidity.

In general the aromatic drugs are slightly fried in order to enhance their aroma and milling properties. Any extraneous material, organic or inorganic, should be removed from the drugs by close inspection.

The chooranam should be so fine to be called amorphous and should never be damp. The fineness of the sieve should be 100 mesh or still finer.

Purification of the prepared chooranam:

"தானென்ற சூரணத்தின் சுத்திக்கேளு

தப்பாதே சரக்கெல்லாஞ் சூரணித்து

நானென்ற வாவின்பாலாற் பிசைந்து

நலமான சட்டியிலே பாலைவிட்டு

வானென்ற சுத்தசலம் பாதிவிட்டு

வளமாக மேற்சீலை கோடுகட்டிப்

பானென்ற சூரணத்தைப் பிட்டுபோல் வைது

பதறாதே வெந்தெடுக்க்ச் சித்தியமே!"

-அகஸ்தியர் வைத்திய இரத்தினச்சுருக்கம்[43]

The prepared chooranam is mixed with the milk in pot half quantity milk and half a quantity water is taken. The mouth of the pot is covered with a thin cloth material. Above this cloth the mixed chooranam is placed. The pot is placed over the stove and heated.

"ஆமப்பா ரவியுலர்த்திப்பொடி தான்செய்து

அப்பனே சமனாய்ச் சர்க்கரையைச்சேர்த்து

நாமப்பா கொண்டுவர தோஷம்போச்சு

நன்றாக்ச் சுத்திசெய்யாச் சூரணந்தான்

தாமப்பா ரோகத்தை வெல்லாதப்பா

தளமான வியதியெல்லாம் பாரிக்கும்பார்

வேமப்பா சுத்திசெய்து கொண்டாயனால்

வெகுசுறுக்காய் தீருமா வியாதிகேளு"

-அகஸ்தியர் வைத்திய இரத்தினச்சுருக்கம்

Then the chooranam is placed in the sunlight and powdered. All type of diseases gets cured. If the drug is taken without purification, the disease does not cure. If taken after purification the disease cures easily.

Storage:

The prepared chooranam should be allowed to cool by spreading and mixing, prior to packing. They should be stored in tightly stoppered glass, polythene or tin containers, or in polythene or cellophane bags and sealed. These bags should in turn be enclosed in cupboard boxes.

The chooranam to facilitate easy handling and to assure exact dosage administration, could be pressed into tablets, could be packed in bottles or tubes made either of glass or plastic or packed in strip of metal foil or plastic sheets.

In industry the tablets are made, counted and packed by electronic devices.

Then chooranam is said to retain its potency for 3 months and then gradually deteriorate. However if properly packed and stored, they keep good for a year. (Formulary of Siddha Medicines, 1993)[42a]

According to AYUSH guidelines shelf life of chooranam is one year.[44]

Table 6: ANALYTICAL SPECIFICATIONS OF CURNA/CHOORNAM

Sl.No	TESTS
1.	Description Macroscopic, Microscopic
2.	Loss on drying at 105˚ C
3.	Total – ash
4.	Acid – insoluble ash
5.	Water-soluble extractive
6.	Alcohol – soluble extractive
7.	Particle size (80-100 mesh for Churna; 40-60 mesh for churna)
8.	Identifications,HPLC-withmarker(wherever possible) Test for heavy/Toxic metals

9.	Lead Cadmium Mercury Arsenic Microbial contamination
10.	Total bacterial count Total fungal count Test for specific Pathogen
11.	E. coli Salmonella spp. S.aureus Pseudomonas aeruginosa Pesticide residue
12.	Organochlorine pesticides Organophosphorus pesticides Pyrethroids Test for Aflatoxins (B1,B2,G1,G2)

3.7.LATERAL RESEARCH

1. ***Elettaria cardamomum:***

Anti-ulcerogenic activity of *Elettaria cardamomum*

The gastro protective action of petroleum ether soluble fractions and essential oils of *E.cardamomum* is due to increase in gastric motility and it has inhibitory effect in over production of some products of 5-lipoxygenase pathway [45].

Anti-convulsant activity:

The methanolic extract of *E.cardamomum* against chemically (pentylentetrazole) and electrically (maximal electric shock) induced seizures in mice. Various pharmacological activities Anti -inflammatory, Analgesic, Anti -oxidant, Anti- microbial effects [46].

2. Nelumbo nucifera:

Cardioprotective effect of Nelumbo nucifera flower extract against isoproterinol induced oxidative stress in male albino Swiss rats

The present study investigates the cardioprotective effect of *Nelumbo nucifera* flower in isoproterinol induced rats. The positive hypertrophy response of isoproterinol caused a severe oxidative stress in the myocardium through increased lipid peroxidation. *Nelumbo nucifera* was administered intra peritonealy at a dose of 200mg/kg for a period of 30days.[47]

Psycopharmacological activity:

Methanolic extract of rhizomes of *Nelumbo nucifera* was investigated for different psychopharmacological action in rats and mice. The extract was found to cause reduction in spontaneous activity, decrease in exploratory behavioral pattern by the head dip and Y-maze test, reduction in the muscle relaxant activity by rotarod 30^0 inclined screen and traction test and potentiated the pentobarbitone induced sleeping time in mice significantly[47a].

3. Nymphaea nouchali

Nymphaea nouchali possesses Antibacterial, Anti-proliferative, Anti-microbial, Anti-Diabetic also been studied [48].

4. *Cyperus rotundus*

Anti-diabetic activity of hydroethsnolic extract of Cyperus rotundus in alloxon induced diabetic rats.

In right of the traditional claim of Cyperus rotundus in the treatment of diabetes, investigations were carried out to evaluate its effect on alloxan induced hyperglycemia in rats. Oral daily administration of 500 mg/kg of the extract (once a day for seven consecutive days) significantly lowered the blood glucose levels [48].

5. *Syzygium aromaticum*

Syzygium aromaticum possesess inhibitory activity against oral pathogens, Anti-oxidant property, anti-fungal, Immediate hypersensitivity in rats, Antimutagenic activity, Cytotoxicity to human skin cells, Fungicidal activity also been studied[49].

6. *Ziziphus mauritiana*

Protective effect of Ziziphus mauritiana leaf extract on carbon tetrachloride-induced liver injury.

Pretreatment of rats with 200 and 300 mg/kg body wt of Z.mauritiana leaf extract protected rats against carbon tetrachloride liver injury by significantly lowering aspartate aminotransaminase (AST), alanine aminotransamine (ALT), alkaline phosphatase (ALP), total bilirubin (TB), and lipid peroxide levels compared to control [50].

7. Glycyrrhiza glabra:

Glycyrrhiza glabra possesses Anti-bacterial, Anti -oxidant, Anti -malarial, Anti spasmodic, Anti -inflammatory, Anti hyperglycemic properties. Various other effects like ulcer, Anti-viral, Hepatotoxic, and Anti- fungal have also been studied [51].

Memory enhancing activity:

The dose of 150 mg/kg of the aqueous extract of liquorice significantly improved learning and memory of mice [52].

8. *Oryza sativa*

Application of brassinosteroids to seeds on growth, pigment levels and nitrate reductase activity of rice (Oryza sativa L) plants grown on saline substratum was investigated. Brassinosteroids reduced the impact of salt stress on growth, considerabley restored pigment levels and increased of nitrate reductase activity [53].

Anti-oxidant activity, Scavenging of reactive oxygen species in NaCl-stressed rice, Influence of N and Ni supply on nitrogen metabolism and urease activity in rice also been studied.

9. *Dryobalanops aromatica*

Nematicidal and ovicidal activity, Genetic diversity, Hepatoprotective activity, Inhibition of acetylcholine-mediated effects by borneol, Inhibitory activities of polyphenols, Synthesis and cytotoxic activity of 3, 4, 11-triydroxyl modified derivatives of begenin also been studied [49a].

4. MATERIALS AND METHODS

4.1 Drug selections

In this dissertation purified and prepared "***Eladhi chooranam"*** was taken as a trial drug for Anti-Hypertensive activity from the Siddha Literature ***"Sarabenthirar Vaithya Muraigal "***authored by **Dr.S.Venkatarajan (L.I.M)**[54]

Table 7: Ingredients of *Eladhi Chooranam:*

S.NO	TAMIL NAME	BOTANICAL NAME	PART USED
1.	*Elakkai*	*Elettaria cardamomum*	Seeds
2.	*Thamarai poovithaz*	*Nelumbo nucifera*	Dried Flower
3.	*Allipoo*	*Nymphaea nouchali*	Dried Flower
4.	*Korai kizhangu*	*Cyperus rotendus*	Rhizome
5.	*Adhimathuram*	*Glycyrrhiza glabra*	Root
6.	*Kirambu*	*Syzygium aromaticum*	Flower
7.	*Ilanthai kottai paruppu*	*Ziziphus mauritiania*	Seed
8.	*Pachai karpooram*	*Dryobalanops aromatica*	Supliment
9.	*Nerpori*	*Oryza sativa*	Fried paddy

Quantity of Ingredients:

- Elakkai *(Elettaria cardamomum)*
- Thamarai poovithaz *(Nelumbo nucifera)*
- Allipoo *(Nymphaea nouchali)*
- Korai kizhangu *(Cyperus rotundus)*
- Athimathuram *(Glycyrrhiza glabra)*
- Kirambu *(Syzygium aromaticum)*
- Ilanthai kottai paruppu *(Ziziphus mauritiania)*
- Pachai karpooram *(Dryobalanops aromatica)*
- Nerpori *(Oryza sativa)*

Equal ratio

Collection of the Plant materials

- The raw materials of Elettaria cardamomum, Dry Cyperus rotundus , Glycyrrhiza glabra root, Syzygium aromaticum, Dry Ziziphus mauritiania, Dryobalanops aromatica and Oryza sativa were collected from the raw drug country shop at Parrys corner, Chennai, Tamilnadu.
- The Petals of Lotus were collected at Sriperumbudur.
- The Petals of Lilly were collected at Kancheepuram.

Identification and Authentication of the drug

All the plant materials were identified and authenticated by the Gunapadam experts in Government Siddha Medical College, Arumbakkam, and Chennai – 106. The specimen sample of all the herbs have been preserved in PG *Gunapadam* department individually for future reference.

Purification of the drugs

All the drugs mentioned here were purified as per the Siddha literature. [55]

Elakkai:

Fried at low flame.

Korai kizhangu:

Impurities are removed and fried at low flame.

Adhimdhuram:

The root of Indian liquorice was cleaned with water and cut into small pieces and then dried.

Kirambu:

Fried at low flame.

Ilanthai kottai paruppu:

Impurities are removed and the outer shell was breaked off.

Pachai karpooram:

Impurities are removed.

Nerpori:

Impurities are removed.

Thamarai Poo Ithalkal:

Impurities are removed and dried in cool dark place.

Allipoo:

Impurities are removed and dried in cool dark place.

Preparation of the Drug

Procedure

All the above-mentioned ingredients were purified and dried in the shade until complete evaporation of the moisture content. It was roasted and powdered and filtered individually. (Fine process). Then all are thoroughly mixed to make *Eladhi Chooranam* and kept in an air tight container. It was labelled as "***Eladhi Chooranam" (EC).***

Purification of the chooranam:

Steaming process (*Pittaviyalmurai*)

The ***Eladhi Chooranam*** was purified by *pittaviyal* method (steam cooking in milk) as per Siddha classical literature. A mud pot was taken and it was half filled by mixture of milk with equal quantity of water. The mouth of the pot was sealed by a cloth. This *chooranam* was placed over the cloth and tied firmly around the mouth of mud pot by another pot. The gap between mud pots was tied with a wet cloth to avoid evaporation. The mud pot was kept on fire and boiled until the cow's milk 3/4 reduced in the lower pot. The same drug was later dried and powdered then sieved again. It was used for the further study [56].

Storage of the drug

The prepared test drug was stored in a clean, air tight glass container. The contents were inspected frequently to avoid moisture and insects.

Administration of the drug

Form of the medicine	:	*Chooranam*
Route of Administration:		Enteral
Dose	:	5.1 g twice a day
Vehicle	:	Honey
Indications	:	Kuruthi Azhal Noi (Hypertension)

Ingredients of Eladhi *Chooranam:*

Thamarai poovithaz
Nelumbo nucifera
Fig no 1.1

Korai kizhangu
Cyperus rotendus
Fig no 1.2

Elakkai
Elettaria cardamomum
Fig no 1.3

Allipoo
Nymphaea nouchali
Fig no 1.4

Adhimathuram
Glycyrrhiza glabra
Fig no 1.5

Kirambu
Syzygium aromaticum
Fig no 1.6

Ilanthai kottai paruppu
Ziziphus mauritiania
Fig no 1.7

Pachai karpooram
Dryobalanops aromatic
Fig no 1.8

Nerpori
Oryza sativa
Fig no 1.9

Procedures of Eladhi Chooranam

Pounding of *Eladhi Chooranam*
Fig no 2.1

Sieving *Eladhi Chooranam*
Fig no 2.2

Eladhi Chooranam

Fig no 2.3

4.2 STANDARDIZATION OF THE DRUG

Standardization of the drug brings the validation to be used as a medicine by subjecting the drug to many analysis and determining its quality and effectiveness. Standardization includes many studies such as its organoleptic properties, physical characteristics and phytochemical properties and also to assess the active principles and elements present in the drug. Thus, standardization brings the efficacy and potency of the drug.

4.2.1 ORGANOLEPTIC CHARACTER

The organoleptic characters of the sample drug were evaluated.1gm of the test drug was taken and the colour, odour, taste, texture, particle size and other morphology were viewed by naked eye under sunlight. Then the result was noted [57].

4.2.2 PHYSICOCHEMICAL ANALYSIS

Physico chemical investigations like Solubility, pH value, Loss on drying at 105°C, and Ash test have been done at The Tamilnadu Dr M.G.R Medical University, Anna salai, Guindy, as per the guide lines of WHO [58].

Solubility:

A pinch of sample (*EC*) was taken in a dry test tube and to it 2 ml of the solvent was added and shaken well for about a minute and the results are observed. The test was done for solvents like distilled water, Ethanol, Petroleum ether, Propylene glycol, Toluene, Benzene, Chloroform, Ethyl alcohol, Xylene, Carbon tetra chloride and the results are observed individually.

pH value:

Potentiometrically, pH value is determined by a glass electrode and a suitable pH meter. The pH of the *EC* was written in results column.

Loss On Drying:

An accurately weighed 1g of ***Eladhi Chooranam*** formulation was taken in a tarred glass bottle. The crude drug was heated at 105^0C for 6 hours in an oven till a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

Determination of total ash:

Weighed accurately 2g of ***Eladhi Chooranam*** formulation was added in crucible at a temperature 600^0C in a muffle furnace till carbon free ash was obtained. It was calculated with reference to the air dried drug.

Determination of acid insoluble ash:

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble as was calculated with reference to the air dried drug.

Determination of water soluble ash:

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450^0C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

Determination of water soluble Extractive:

5gm of air dried drug, coarsely powered ***Eladhi chooranam*** was macerated with 100ml of distilled water in a closed flask for twenty-four hours shaking frequently. The Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100^0 C and weighted. The percentage of water soluble extractive was calculated with reference to the air dried drugs.

Determination of alcohol soluble extractive:

1 gm of air dried drugs, coarsely powdered ***Eladhi chooranam*** was macerated with 20 ml. alcohol in closed flask for 24 hrs with frequent shaking. It was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100^0C and weighted. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

4.2.3 PHYTOCHEMICAL ANALYSIS

The Phytochemical screening of the extract gives general idea regarding the nature of chemical constituents present in the crude drug. The phytochemical tests were done as the method illustrated in [59].

1. Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

2. Detection of carbohydrates:

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides:

Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

a) **Cardiac glycoside (Keller-Killiani test):** Extract was shaken with distilled water (5 mL). To this, glacial acetic acid (2 mL) containing a few drops of ferric chloride was added, followed by H2SO4 (1 mL) along the side of the test tube. The formation of brown ring at the interface gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

5. Detection of phenols Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6. Detection of tannins Gelatin Test:

The extract is dissolved in 5 ml of distilled water and 2 ml of 1% solution of Gelatin containing 10% NaCl is added to it. White precipitate indicates the presence of phenolic compounds.

7. Detection of Flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodiumhydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

8. Detection of proteins

a) Xanthoprotein Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

9. Detection of aminoacids

a) Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

10. Detection of diterpenes Copper Acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

11. Gum and Mucilage:

To 1ml of extract add 2.5ml of absolute alcohol and stirring constantly. Then the precipitate was dried in air and examine for its swelling properties. Swelling was observed that will indicate presence of gum and mucilage.

12. Test for Quinones

Extract was treated with sodium hydroxide blue or red precipitate indicates the presence of Quinones.

13. Test for Fixed oils and Fats

a. Spot test: A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.

HPLC - High Performance Liquid Chromatography (HPLC) [60]

HPLC is a technique in analytical chemistry which is used to separate the components in a mixture, to identify each component, and to quantify each component. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. In this study, the detection and quantitation were carried out using 515 HPLC pumps and 2489 UV/Visible detectors of Waters Company while the software used was Empower.

Two methods using different mobile phases were used for chromatographic separation of the research drugs – Method I (binary gradient method of Acetonitrile & 0.1% Phosphoric acid in Water) and Method II (binary gradient method of Methanol & 1:25 Acetic acid in Water). Results obtained during Method I have been discussed since better separation of compounds was observed during this analysis. The chromatographic conditions for Method I are as given below:

Column	: Symmetry C18, 5µm, 4.6x250 mm
Run Time	: 30 minutes
Injection Volume	: 20 µl
Wavelength (Dual)	: 272 nm & 360 nm
Solvent A	: Acetonitrile
Solvent B	: 0.1% Phosphoric acid in water
Flow rate	: 1.0 ml/min.
Pump Mode	: Gradient

4.2.4 BIO-CHEMICAL ANALYSIS

Preliminary Basic and Acidic radical studies [61]

Preparation of extract

5gm of *EC* was taken in a 250 ml of clean beaker and 50 ml of distilled water was added to it. Then it was boiled well for about 10 minutes. Then it was allowed to cool and filtered in a 100 ml volumetric flask and made up to 100 ml with distilled water. This preparation was used for the qualitative analysis of acidic/ basic radicals and biochemical constituents in it.

Test for Basic radicals

1. Test for Potassium

To a pinch of the *EC*, 2 ml of sodium nitrate and 2 ml of cobalt nitrate solution in 30% glacial acetic acid was added and observed for the presence of yellow precipitate.

2. Test for Calcium

To 2 ml of *EC* extract, 2 ml of 4% ammonium oxide solution was added and observed for the formation of white precipitate**.**

3. Test for Magnesium:

To 2ml of *EC* extract, drops of sodium hydroxide solution was added and watched for the appearance of white precipitate.

4. Test for Ammonium:

To 2ml of *EC* extract few ml of Nessler's reagent and excess of sodium hydroxide solution are added for the appearance of brown colour.

5. Test for Sodium

Hydrochloric acid was added with a pinch of the *EC*, made as paste and introduced into the blue flame of Bunsen burner and observed for the appearance of intense yellow colour.

6. Test for Iron (Ferrous)

The *EC* extract was treated with Conc. HNO_3 and ammonium thiocyanate and waited for the appearance of blood red colour**.**

7. Test for Zinc

To 2 ml of the *EC* extract drops of sodium hydroxide solution was added and observed for white precipitate formation.

8. Test for Aluminium

To the 2m1 of the *EC* extract sodium hydroxide was added in drops and changes are noted.

9. Test for Lead

To 2 ml of *EC* extract 2ml of potassium iodide solution was added and noted for yellow coloured precipitate**.**

10. Test for Copper

a. A pinch of *EC* was made into a paste with con. Hcl in a watch glass and introduced into the non-luminous part of the flame and noted for blue colour appearance.

b. To 2 ml of *EC* extract excess of ammonia solution was added and observed for the appearance of blue coloured precipitate**.**

11. Test for Mercury

To 2m1 of the *EC* extract sodium hydroxide solution was added and noted for yellow precipitate formation**.**

12. Test for Arsenic

To 2 ml of the *EC* extract 2ml of sodium hydroxide solution was added and brown or red precipitate formation was noted.

Test for acid radicals

1. Test for Sulphate

To 2 ml of the *EC* extract 5% of barium chloride solution was added and observed for the appearance of white precipitate.

2. Test for Chloride

The *EC* extract was treated with silver nitrate solution and observed for the appearance of white precipitate.

3. **Test for Phosphate**

The *EC* extract was treated with ammonium molybdate and conc. HNO_3 and observed for the appearance of yellow precipitate.

4. **Test for Carbonate**

The *EC* extract was treated with conc.Hcl and observed froth appearance of effervescence indicate the presence of carbonate.

5. Test for Fluoride & Oxalate:

To 2ml of *EC* extract 2ml of dil.acetic acid and 2ml calcium chloride solution was added and heated and watched for cloudy appearance.

6. Test for Nitrate:

To 1 gm of the *EC*, copper turnings was added and again conc.H_2SO_4 was added, heated and the test tube was tilted vertically down and observed for any changes.

4.2.5 MICROBIAL ACTIVITY:

AGAR- WELL DIFFUSION METHOD [62]

PRINCIPLE

The antimicrobials present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters.

MATERIALS REQUIRED

1Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.8 g of the commercially available Muller Hinton Agar Medium (MHI Agar Media) in 1000ml of distilled water. The dissolved

medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HI Media) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

1. *E.coli* (ATCC 25922)
2. *Staphylococcus aureus* (ATCC 25923)
3. *Pseudomonas aeroginosa* (ATCC 27853)
4. *Klebsiella pneumoniae* (ATCC 13883)

PROCEDURE

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E.coli*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae a*nd *Staphylococcus aureus* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of sample such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). Streptomycin was used as a positive control.

ANTIFUNGAL ACTIVITY[63]

AGAR- WELL DIFFUSION METHOD

PRINCIPLE

In order to access the biological significance and ability of the sample, the antifungal activity was determined by Agar well diffusion method. The antifungals present in the samples are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in **millimeters**.

MATERIALS REQUIRED

1) Potato Dextrose Agar Medium (1 L)

The medium was prepared by dissolving 39 g of the commercially available Potato Dextrose Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

3. Clotrimazole (standard antifungal agent, concentration: 10mg / ml)

4. Culture of test organisms; growth of culture adjusted according to McFarland Standard, 0.5%

- *Aspergillus niger* (ATCC 16404*)*

PROCEDURE

Potato Dextrose agar plates were prepared and overnight grown species of fungus, *Aspergillus niger* were swabbed. Wells of approximately 10mm was bored using a well cutter and samples of different concentrations such as 250µg/mL, 500µg/mL and 1000µg/mL were added. The zone of inhibition was measured after overnight incubation at room temperature and compared with that of standard antimycotic (Clotrimazole) (NCCLS, 1993).

4.2.6 INSTRUMENTAL ANALYSIS

SOPHISTICATED INSTRUMENTAL ANALYSIS

FT IR - Fourier Transform Infra-red Spectroscopy [64]

FTIR (Fourier Transform Infra-red Spectroscopy) is a sensitive technique particularly for identifying organic chemicals in a whole range of applications although it can also characterise some inorganics. Examples include paints, adhesives, resins, polymers, coatings and drugs. FTIR is an effective analytical instrument for detecting functional groups.

Fig no 3.1: FTIR INSTRUMENT

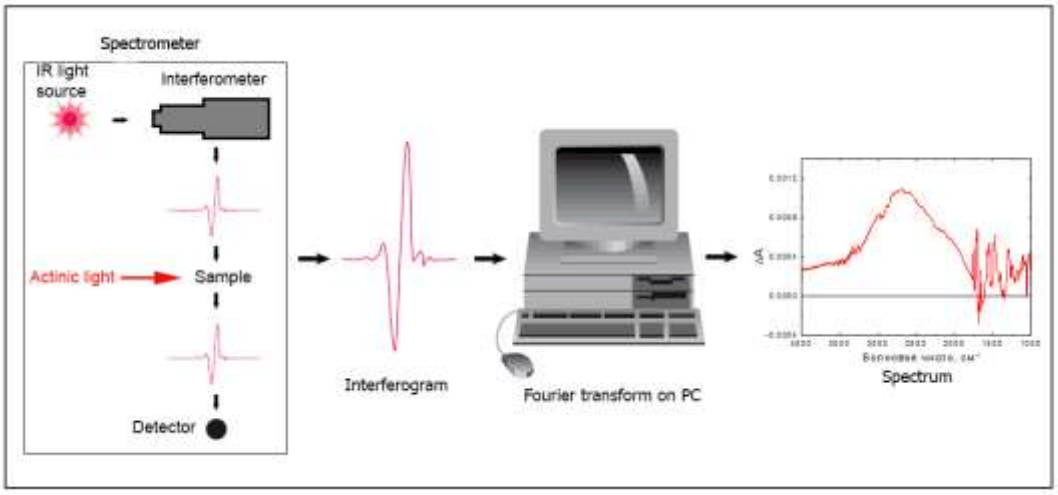


Fig no 3.2: FTIR MECHANISM

APPLICATIONS:

- Quantative scans
- Qualitative scan solids, liquids, gasses
- Organic samples, inorganic samples
- Unknown identification
- Impurities screening
- Formulation
- Pharmaceuticals

Principle:

Spectrophotometric tests are commonly used in the Identification of chemical substances and quantification of polymorphic forms. The test procedures are applicable to substances that absorb IR radiation. The IR absorption spectrum of a substance compared with that obtained concomitantly for the corresponding reference standard / reference substance provide conclusive evidence of the identity of the substance being tested.

Recording Infrared spectrum of a solid as a disc (as per USP ‹197K›):

- Triturate about 1 to 2 mg of the substance to be examined with 300 to 400 mg, unless otherwise specified, of finely powdered and dried potassium bromide. If the substance is a hydrochloride it is preferable to use potassium chloride.
- Carefully grind the mixture and spread it uniformly in a suitable die.
- Submit it to the pressure of about 800 mPa (8 tons/ cm^2).
- Examine the disc visually and if any lack of uniform transparency is observed, reject the disc and prepare again.
- Record the spectrum between 4000 to 650 cm-1 unless otherwise specified in individual standard test procedure.
- When sample and standard are measured for concordance, the transmittance obtained at the start of the scan range, should not deviate by more than 10% between them (For eg. If the standard shows a transmittance of 75%, the sample transmittance can be between 65% and 85%).

FT-IR was the most advanced and the major advantage was its

- Speed
- Sensitivity
- Mechanical Simplicity
- Internally Calibrated

ICPOES (INDUCTIVELY COUPLED PLASMA OPTIC EMISSION SPECTROMETRY)

Fig no 4.1 ICPOES Instrument

Manufacturer: Perkin Elmer

Model: Optima 5300 DV ICP-OES Inductively Coupled Plasma Spectrometer (ICP)

Principle:

An aqueous sample was converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma which was a high temperature zone (8,000– 10,000ºC). The analysts are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions (lights). These releases are separated based on their respective wavelengths and their strengths are measured (spectrometry). The intensities are proportional to the concentrations of analyses in the aqueous sample. The quantification was an external multipoint linear standardization by comparing the emission intensity of an unknown sample with that of a standard sample. Multi-element calibration standard solutions are prepared from single- and multi element primary standard solutions. With respect to other kinds of

analysis where chemical speciation was relevant (such as the concentration of ferrous iron or ferric iron), only total essential concentration was analysed by ICP-OES. [65]

Application:

The analysis of major and minor elements in solution EC

Objectives:

- Determine elemental concentrations of different metals.
- Learn principles and operation of the ICP-OES instrument
- Develop and put on a method for the ICP-OES sample analysis
- Enhance the instrumental conditions for the analysis of different elements
- probes the outer electronic structure of atoms

Mechanism:

In plasma emwassion spectroscopy (OES), a ECM solution was presented into the core of inductively coupled argon plasma (ICP), which generates temperature of approximately 8000°C. At this temperature all elements become thermally excited and emit light at their characteristic wavelengths. This light was collected by the spectrometer and passes through a diffraction grating that serves to resolve the light into a spectrum of its essential wavelengths. Within the spectrometer, this deflected light was then collected by wavelength and amplified to yield an strength of measurement that can be converted to an elemental concentration by comparison with standardization values.[66]

The Inductively coupled plasma optical emission spectrometric (ICP-OES) analysis was done in SAIF, IIT MADRAS, and Chennai-36 using Perkin Elmer Optima 5300 DV.

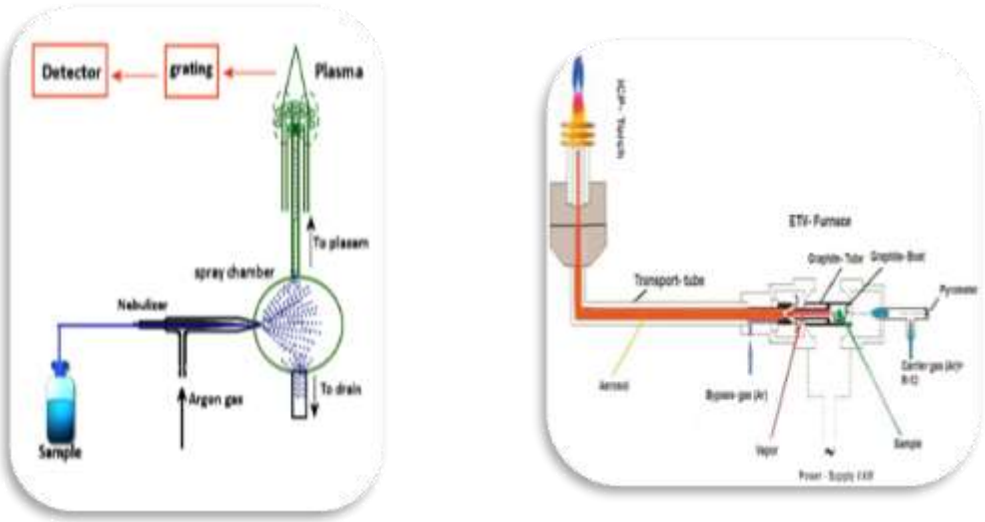


Fig no 4.2 ICPOES Mechanism

SEM - Scanning Electron Microscope [67]

DEFINITION

Scanning Electron Microscopy (SEM), also known as SEM analysis or SEM microscopy, is used very effectively in microanalysis and failure analysis of solid inorganic materials. Scanning electron microscopy is performed at high magnifications, generates high-resolution images and precisely measures very small features and objects.

SEM ANALYSIS APPLICATIONS

The signals generated during SEM analysis produce a two-dimensional image and reveal information about the sample including:

External morphology (texture)

- Chemical composition (when used with EDS) Orientation of materials making up the sample

The EDS component of the system is applied in conjunction with SEM analysis to:

- Determine elements in or on the surface of the sample for qualitative information
- Measure elemental composition for semi-quantitative results
- Identify foreign substances that are not organic in nature and coatings on metal
- SEM Analysis with EDS – qualitative and semi-quantitative results
- Magnification – from 5x to 300,000x
- Sample Size – up to 200 mm (7.87 in.) in diameter and 80 mm (3.14 in.) in height
- Materials analysed – solid inorganic materials including metals and minerals.

Fig no 5.1 SEM INSTRUMENT

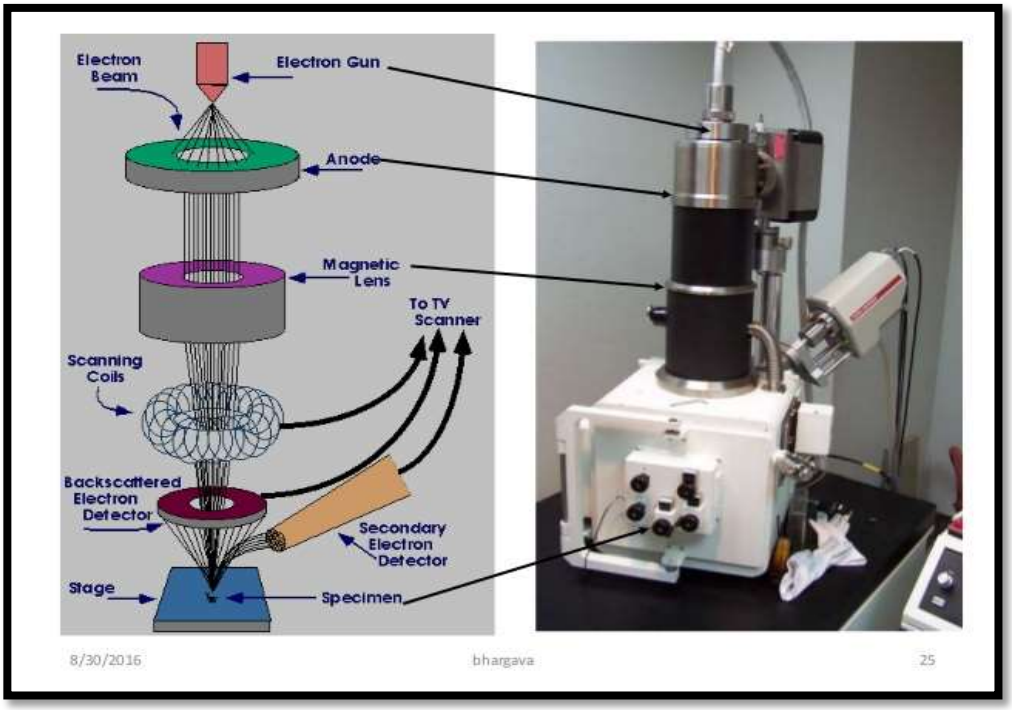


Fig no: 5.2 SEM MECHANISMS

THE SEM ANALYSIS PROCESS

Scanning Electron Microscopy uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. In most SEM microscopy applications, data is collected over a selected area of the surface of the sample and a two-dimensional image is generated that displays spatial variations in

properties including chemical characterization, texture and orientation of materials. The SEM is also capable of performing analyses of selected point locations on the sample. This approach is especially useful in qualitatively or semi-quantitatively determining chemical compositions, crystalline structure and crystal orientations.

The EDS detector separates the characteristic X-rays of different elements into an energy spectrum and EDS system software is used to analyse the energy spectrum in order to determine the abundance of specific elements. A typical EDS spectrum is portrayed as a plot of X-ray counts vs. energy (in keV). Energy peaks correspond to the various elements in the sample. Energy Dispersive X-ray Spectroscopy can be used to find the chemical composition of materials down to a spot size of a few microns and to create element composition maps over a much broader raster area. Together, these capabilities provide fundamental compositional information for a wide variety of materials, including polymers. In scanning electron microscope high-energy electron beam was focused through a probe towards PP. Variety of signals was produced on interaction with the surface of the sample. This results in the emission of electrons or photons and it was collected by an appropriate detector.

The types of signal produced by a scanning electron microscope include:

- Secondary electrons
- Back scattered electrons
- Characteristic x-rays light
- Specimen current
- Transmitted electrons.

This gives the information about the sample and it includes external morphology, texture, its crystalline structure, chemical composition and it displays the shape of the sample.

XRD - X-ray Powder Diffraction (XRD) [68]

X-ray powder diffraction (XRD) is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analysed material is finely ground, homogenized, and average bulk composition is determined.

DEFINITION

X-ray powder diffraction is most widely used for the identification of unknown crystalline materials (e.g. minerals, inorganic compounds). Determination of unknown solids is important to studies in geology, environmental science, material science and biology.

Fig no 6.1: XRD - X-ray Powder Diffraction Instrument

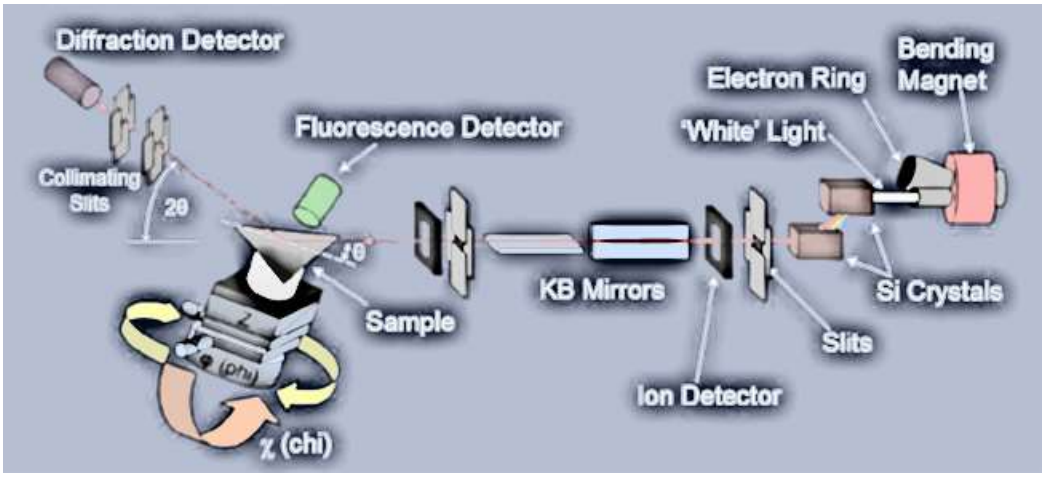


Fig no 6.2 XRD Mechanism

Applications:

- Characterization of crystalline materials
- Identification of fine-grained minerals such as clays and mixed layer clays that are difficult to determine optically
- Determination of unit cell dimensions.

With specialized techniques, XRD can be used to:

- Determine crystal structures using Rietveld refinement
- Determine of modal amounts of minerals (quantitative analysis)
- Characterize thin films samples by:
- Determining lattice mismatch between film and substrate and to inferring stress and strain
- determining dislocation density and quality of the film by rocking curve measurements
- measuring super lattices in multilayered epitaxial structures
- determining the thickness, roughness and density of the film using glancing incidence X-ray reflectivity measurements
- Make textural measurements, such as the orientation of grains, in a polycrystalline sample.

Strengths and Limitations of X-ray Powder Diffraction:

Strengths:

- Powerful and rapid (< 20 min) technique for identification of an unknown mineral
- In most cases, it provides an unambiguous mineral determination
- Minimal sample preparation is required
- XRD units are widely available
- Data interpretation is relatively straight forward.

Limitations:

- Homogeneous and single-phase material is best for identification of unknown
- Must have access to a standard reference file of inorganic compounds
- Requires tenths of a gram of material which must be ground into a powder

- For mixed materials, detection limit is ~ 2% of sample
- For unit cell determinations, indexing of patterns for non-isometric crystal systems is complicated.

Sample Collection and Preparation:

Determination of an unknown requires: the material, an instrument for grinding, and a sample holder.

- Obtain a few tenths of a gram (or more) of the material, as pure as possible
- Grind the sample to a fine powder, typically in a fluid to minimize inducing extra strain (surface energy) that can offset peak positions, and to randomize orientation. Powder less than ~10 ɜm (or 200-mesh) in size is preferred
- Place into a sample holder or onto the sample surface.

4.3 TOXICOLOGICAL STUDIES

4.3.1 ACUTE ORAL TOXICITY STUDY

Acute toxicity study was carried out as per OECD guideline (Organization for Economic Co - operation and Development, Guideline-423 [69].

The experimental protocol was approved by the institutional ethical committee (IAEC) under CPCSEA (IAEC approved number: 04/321/PO/Re/S/01/CPCSEA-12/OCT-2018

These studies were conducted in C.L.Baid Metha College of Pharmacy, Dhuraipakkam, Chennai.

INTRODUCTION:

- The acute toxic class method is a stepwise procedure with the use of 3 animals of a single sex per step.
- Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.
- This procedure is reproducible, uses very few animals and is able to rank substances in a similar manner to the other acute toxicity testing methods.

- The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment.
- In principle, the method is not intended to allow the calculation of a precise LD50 but does allow for the determination of defined exposure ranges where lethality is expected since death of a proportion of the animals is still the major endpoint of this test.
- The method allows for the determination of an LD50 value only when at least two doses result in mortality higher than 0% and lower than 100%.

The use of a selection of pre-defined doses, regardless of test substance, with classification explicitly tied to number of animals observed in different states improves the opportunity for laboratory to laboratory reporting consistency and repeatability.

PRINCIPLE:

It is the principle of the test that based on a stepwise procedure with the use of a minimum number of animals per step, sufficient information is obtained on the acute toxicity of the test substance to enable its classification. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of compound-related mortality of the animals dosed at one step will determine the next step, i.e.;

− No further testing is needed

− dosing of three additional animals with the same dose

− dosing of three additional animals at the next higher or the next lower dose level.

The method will enable a judgment with respect to classifying the test substance to one of a series of toxicity classes.

METHODOLOGY

Selection of Animal Species

The preferred rodent species is the wister rat, although other rodent species may be used. Healthy young adult animals are commonly used laboratory strains

should be employed. Females should be nulliparous and non-pregnant. Each animal, at the commencement of its dosing, should be between 6 to 8 weeks old and the weight (150-250gm) should fall in an interval within±20 % of the mean weight of any previously dosed animals.

Housing and Feeding Conditions

The temperature in the experimental animal room should be 22ºC ± 3ºC. Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning the aim should be 50-60%. Lighting should be artificial, the sequence being 12 hours light, 12 hours dark. For feeding, conventional laboratory diets may be used with an unlimited supply of drinking water. Animals may be group-caged by dose, but the number of animals per cage must not interfere with clear observations of each animal.

Preparation of animals:

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions.

Test Animals and Test Conditions:

Sexually mature Female Wistar albino rats (150-200gm) were obtained from TANUVAS, Madhavaram, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai Meera foods, Bangalore).

PREPARATION OF ACUTE TOXICITY STUDIES:

Rats were deprived of food overnight (but not water 16-18 h) prior to administration of the, ***Eladhi chooranam***.

The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design

IAEC approved Number: 04/321/PO/Re/S/01/CPCSEA-12/OCT-2018

Test Substance	: ***ELADHI CHOORANAM***
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Female-3+3)
Age	**:** 6-8 weeks
Body Weight on Day 0	**:** 150-200gm.

Acclimatization	**:** Seven days prior to dosing.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid.
Number of animals	: 3 Female/group,
Route of administration	: Oral
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.
Housing temperature	**:** between 22ºC ± 3ºC.
Relative humidity	**:** between 30% and 70%,
Air changes	**:** 10 to 15 per hour and
Dark and light cycle	**:** 12**:**12 hours.
Duration of the study	**:** 14 Days

Administration of Doses:

EC was suspended in water and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 3mg/kg body weight was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration.

The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hrs and these animals were then monitored for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

Limit test

The limit test was primarily used in situations where the experimenter has information indicating that the test material was likely to be nontoxic, i.e., having toxicity only above regulatory limit doses. A limit test at one dose level of 2000 mg/kg body weight was carried out with three animals per step.

The test substance-related mortality was not produced in animals, so further testing at the next lower level need not be carried out.

Observations

- The animals were observed individually after dosing at least once during the first 30mins and periodically during the first 24 hours.
- Special attention: First 1-4 hours after administration of drug.
- It was observed daily thereafter for a total of 14 days, except when they needed to be removed from the study and killed humanely for animal welfare reasons or are found dead.

a. Mortality

Animals will be observed intensively at 0.5, 2.0, 4.0, 6.0, 12.0, 24.0 and 48.0 hour following drug administration on day 1 of the experiment and daily twice thereafter for 14 days.

b. Body weight

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

c. Cage-side observation

These include changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour patterns. Attention should be directed to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

d. Gross necropsy

All animals (including those which die during the test period are removed from the study) will be subjected to gross necropsy. Gross necropsy includes examination of the external surface of the body, all orifices, cranial, thoracic and

abdominal cavities and their contents, brain, eye, thymus, lungs, heart, spleen, liver, kidneys, adrenals, testes and uterus of all animals.

Histopathology

Microscopic examination will be carried out in organs to show the evidence of any toxicity in gross pathology.

Data and reporting

All the data were summarised in tabular form, table showing the animals used, number of animals displaying signs of toxicity, the number animals found dead during the test or killed for humane reasons, a description and the time course of toxic effects and reversibility, and necroscopic findings.

Test substance and Vehicle

In order to ensure the uniformity in drug distribution in the medium the suspension was made by mixing *EC* with 2% CMC solution and it was found suitable for dose accuracy.

Justification for choice of vehicle

The vehicle selected as per the standard guideline is pharmacologically inert and easy to employ for new drug development and evaluation technique [70].

4.3.2 REPEATED DOSE 28 DAYS ORAL TOXICITY STUDY OF *VEN THAMARAIYATHI CHOORANAM* ON RATS – (OECD-407 guidelines) [71]

Test Substance	: **ELADHI CHOORANAM**
Animal Source	: TANUVAS, Madhavaram, Chennai.
Animals	: Wister Albino Rats (Male -24, and Female-24)
Age	**:** 6-8 weeks
Body Weight	**:** 150-200gm.
Acclimatization	**:** Seven days prior to dose.
Veterinary examination	**:** Prior and at the end of the acclimatization period.
Identification of animals	**:** By cage number, animal number and individual marking by using Picric acid
Diet	**:** Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore.
Water	**:** Aqua guard portable water in polypropylene bottles.
Housing & Environment	**:** The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature **:** between 22ºC ± 3ºC.

Relative humidity **:** between 30% and 70%.

Air changes **:** 10 to 15 per hour

Dark and light cycle **:** 12**:**12 hours.

Duration of the study **:** 28 Day

Table no 8: Animals and groups

Groups	**No of Rats**
Group I Vehicle control (Water)	12(6male,6 female)
Group II EC- low dose X (20mg)	12 (6male,6 female)
Group III EC- Mid dose 5X (100mg)	12 (6male,6female)
Group IV EC- High dose 10X (200 mg)	12(6male,6female)

Justification for Dose Selection:

The results of acute toxicity studies in Wistar albino rats indicated that *(EC)* was non-toxic and no behavioural changes was observed up to the dose level of 2000 mg/kg body weight. On the basis of body surface area ratio between rat and human, the doses selected as per OECD guideline three dose levels were selected for the study. They are low dose (5X), high dose (10X). X is calculated by multiplying the acute toxicity dose 2000mg) and the body surface area of the rat (0.018), 5X dose is (100mg/kg), 10X dose is (200mg/kg) The oral route was selected for use because oral route is considered to be a proposed therapeutic route.

Preparation and Administration of Dose:

Eladhi chooranam suspended in prescribed medium, it was administered to animals at the dose levels of X, 5X, 10X. The test substance suspensions were freshly prepared every two days once for 28 days. The control animals were administered vehicle only. The drug was administered orally by using oral gavage once daily for 28 consecutive days.

METHODOLOGY

Randomization, Numbering and Grouping of Animals

48 Wistar Albino Rats (24M + 24F) were selected and divided into 4 groups. Each group consist of 12 animals (Male -6, and Female-6). First group treated as a control and other three groups were treated with test drug (low, mid, high) for 28

days. Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the initiation of treatment. Each animal was marked with picric acid. The females were nulliparous and non-pregnant.

OBSERVATIONS

Experimental animals were kept under observation throughout the course of study for the following:

Body Weight:

Weight of each rat was recorded on day 0, at weekly intervals throughout the course of study.

Food and water Consumption:

Food and water consumed per animal was calculated for control and the treated dose groups.

Clinical signs:

All animals were observed daily for clinical signs. The time of onset, intensity and duration of these symptoms, if any, were recorded.

Mortality:

All animals were observed twice daily for mortality during entire course of study.

Functional Observations:

At the end of the 4^{th} week exposure, 'sensory reactivity' to graded stimuli of different types (auditory, visual and proprioceptive stimuli), 'motor reactivity' and 'grip strength' were assessed.

Laboratory Investigations:

Following laboratory investigations were carried out on day 29 in animal's fasted over-night. Blood samples were collected from orbital sinus using sodium heparin (200IU/ml) for Bio chemistry and potassium EDTA (1.5 mg/ml) for Haematology as anticoagulant. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes.

Haematological Investigations:

Blood samples of control and experimental rats was analyzed for hemoglobin content, total red blood corpuscles (RBC), white blood corpuscles (WBC) count and packed cell volume (PCV).

Biochemical Investigations:

Serum was used for the estimation of biochemical parameters. Samples of control and experimental rats were analyzed for protein, bilirubin, urea, BUN, creatinine, triglyceride, cholesterol and glucose levels was carried using standard methods. Activities of glutamate oxaloacetate transaminase/Aspartate aminotransferase (GOT/AST), glutamate pyruvate transaminase/ Alanine amino transferase (GPT/ALT) and alkaline phosphatase were estimated as per the colorimetric procedure.

Necropsy:

All the animals were sacrificed by excessive anaesthesia on day 29. Necropsy of all animals was carried out.

Histopathology:

Control and highest dose group animals will be initially subjected to histopathological investigations. If any abnormality found in the highest dose group than the low, then the mid dose group will also be examined. Organs will be collected from all animals and preserved in 10% buffered neutral formalin for 24 h and washed in running water for 24 h. The organ sliced 5 or 6µm sections and were dehydrated in an auto Technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin red.

Statistical analysis:

Findings such as body weight changes, water and food consumption, and haematology and blood chemistry were subjected to One-way ANOVA followed by dunnet-t test using a computer software programme – Graph pad version 7. All data were summarized in tabular form, (Table-24 to 33)

4.4 PHARMACOLOGICAL STUDIES

4.4.1 ANTI-HYPERTENSIVE ACTIVITY OF *ELADHI CHOORANAM* IN SPOTANEOUSLY HYPERTENSIVE RATS

Cardiovascular disease is a leading cause of death, and hypertension is a critical risk factor for cardiovascular events. The pathogenesis of hypertension is accompanied by decreased nitric oxide (NO) bioavailability in the vasculature and increased cardiovascular remodelling. Hypertensive patients frequently develop clinically evident cardiac hypertrophy 10 to 20 years after the onset of hypertension, as a result of adaptive and maladaptive responses to pressure overload. Cardiac hypertrophy has been linked to the development of a variety of cardiovascular diseases, including myocardial ischemia, arrhythmias, and sudden cardiac death. Therefore, treatment options that not only maintain stable pressure levels but also delay or even regress the structural and functional changes in resistance arteries and the heart are needed. Despite the current availability of multiple anti-hypertensive medication types, a significant number of patients do not respond to treatment and remain hypertensive. As multiple mechanisms likely contribute to the development of hypertension, including angiotensin, oxidative stress and hemodynamic changes, multi-targeted therapeutic interventions will likely be required for effective management of hypertension.

Animals:

All animal experiments were performed in accordance with the Guidelines of OECD. All experiments were performed with the approval of IAEC of C.L. BAID METHA COLLEGE OF PHARMACY. SHR (9 weeks old) and age-matched Wistar rats male, weighing 250±20 g, were purchased from King Institute of Preventive Medicine and Research, Rats were kept in a room temperature-controlled room (25 °C), with 12 hours dark and 12 hours artificial illumination daily (7:00— 19:00). Food and water were available ad libitum.

GROUPING

The animals were divided into following groups:

- Group 1 control untreated group which received normal saline.
- Group 2 received Verapamil 12.5 mg/kg b.w
- Group 3 *EC* 200 mg/kg b.w
- Group 4 *EC* 400mg/kg b.w

The drug *EC* was administered orally and once daily for 4 weeks.

In this study, the effect of a four weeks chronic administration of daily oral doses of 200 and 400 mg/kg body weight, *EC* on blood pressure was measured.

The stock solution was prepared once every three days. Extract suspensions were stored at 4ºC and were allowed to reach room temperature before administration.

METHOD:

Systolic blood pressure (SBP) and heart rate measurement of SH rats was carried out using tail-cuff method plethysmography (LE 5001 Pressure Meter). A mean of six measurements was obtained for each animal. For blood pressure measurement, the animals were warmed up to 42°C for 5 min in a confinement cage. The animals were first submitted to a period of adaptation for 15 days before the experiments and only SHR with an SBP > 170 mmHg were selected for this study.

During the final week of the treatment, the rats were allowed to acclimatize to the experimental conditions of non-invasive SBP measurements by allowing them to stand in rat restrainers for 30 min every day. SBP measurements were recorded 24 hours after the last treatment dose. At least 8-10 recordings were taken for each rat and the mean of the lowest 4 values within less than 10 mmHg difference was taken as the mean SBP [72].

4.4.2 DIURETIC ACTIVITY OF *ELADHI CHOORANAM* [73]

ANIMALS

Male Wistar rats (175-200g) were purchased from TANUVAS Chennai. They were maintained under standard conditions of temperature and humidity. The method of Lipschitz et al was employed for the assessment of diuretic activity. FOUR groups of six rats.

GROUPING

The animals were divided into following groups:

- Group 1----control untreated group which received normal saline.
- Group 2 ----received Furosemide12.5 mg/kg b.w
- Group 3-----200mg/kg b.w
- Group 4-----400mg/kg b.w

Each were fasted and deprived of water for eighteen hours prior to the experiment. On the day of experiment, normal group of animals were given normal saline orally (25 ml/kg body weight.) And the treated groups were given 100,200 EC mg/kg bodyweight of rats and water. The standard groups were given furosemide (20mg/kg) intra peritoneal. The rats were placed in metabolic cages specially designed to separate faecal matter and urine. The urine volume was collected at 24 hours post administration. During this period no food or water was given to the animals. The total urine volume was measured for both control and treated animals. The sodium, potassium and chloride ion concentration in the urine samples were determined.

The urine was collected for 5^{th} hour and 24^{th} hour after administration control, standard and test drug. The bladder was emptied by pull the base of tail of each rat[74]

OBSERVATION

- Animals are subjected to collect urine periodically by metabolic cages.
- Diuretic assay parameters were observed for each rat.
- The total volume of urine was measured.
- Urinary pH, urinary sodium excretion, urinary potassium excretion, urinary chloride excretions are determined. The concentration of sodium,

potassium and chloride levels excreted in the urine were measured by flame photometry and the chloride concentration was estimated by titration with silver nitrate solution(N/50) using 3 drops of 5% potassium chromate as indicator [75]

- The data was analyzed using one-way analysis of variance (ANOVA).
- The statistical significance of the difference of the means was evaluated by Dunnett's multiple comparison test

4.4.3 ANTI-OXIDANT ACTIVITY OF *ELADHI CHOORANAM*

DPPH ASSAY (2, 2-diphenyl -1-picrylhydrazyl) IN-VITRO [76]

The radical scavenging activity of different extracts was determined by using DPPH assay according to [74] Chang et al [2001]. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 517 nm.

Ascorbic acid (10mg/ml DMSO) was used as reference.

PRINCIPLE

1,1-diphenyl-2-picrylhydrazyl is a stable free radical with red colour which turns yellow when scavenged. The DPPH assay uses this character to show free radical scavenging activity. The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as,

$$DPPH + [H\text{-}A] \rightarrow DPPH\text{-}H + (A)$$

Antioxidants react with DPPH and reduce it to DPPH-H and as consequence the absorbance decreases. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

REAGENT PREPARATION

0.1ml DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol.

PROCEDURE

Different volumes (1.25-20µg/µl) of *EC* extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mm) solution was added. Their action mixture was incubated in dark condition at room temperature for 20min. After 20min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. The % radical scavenging activity of the *EC* extracts was calculated using the following formula,

$$\% \text{ inhibition} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

5. RESULTS AND DISCUSSION

There are so many advanced studies have been carried out to bring the efficacy and potency of the drug "***Eladhi chooranam".*** The study includes literary collections, organoleptic characters, physicochemical, phytochemical analysis, Acid-Base radical test, Microbial load, instrumental analysis, toxicological study and pharmacological study. The drug "***Eladhi Chooranam"*** has been selected for **Anti-Hypertensive** activity in reference with the text ***"Sarabenthirar Vaithya Muraigal ".*** Literary collections about the drug from various text books were done. Siddha literatures related to the drug bring the evidence and importance of its utility in treating the hypertension.

- Botanical aspect explains the identification, description, active principle and medicinal uses of the plants.
- Gunapadam review brings the effectiveness of the drug in treating hypertension.
- The pharmacological review explains about the methodology of Anti-Hypertensive Activity and the drugs used.
- Pharmaceutical review describes about the *chooranam* and its properties.
- Lateral research gives the effectiveness of the drug in treating hypertension.
- Siddha and Modern aspect of the disease was also reviewed.

DISCUSSION ON STANDARDIZATION TECHNIQUES:

STANDARDIZATION OF THE TEST DRUG

Standardization of the drug is more essential to derive the efficacy, potency of the drug by analysing it by various studies. Following are the results of physicochemical and phytochemical analysis; physical characterisation and estimation of basic and acidic radicals have been done and tabulated.

Toxicological results of the drug and pharmacological activity of the drug were derived. Its result has been tabulated and interpretation was made below. Thus, it is to give a complete justification to bring the effectiveness of the trial drug **"*Eladhi Chooranam"***.

ORGANOLEPTIC CHARACTERS

The following characters have been noted in *Eladhi chooranam*.

Table no 9: Organoleptic Characters of *Eladhi chooranam*

Colour	Brown
Odour	Pleasant
Taste	Bitter & sweet
Texture	Fine powder
Particle size	Completely pass through sieve no 88

Discussion:

The organoleptic characters of the drug *Eladhi chooranam* showed yellowish green colour since prepared from dry herbs, Bitter and sweet in taste which might be responsible for the activity mentioned earlier and on sight they are fine powder.

- The fineness of the *chooranam* represents easy absorption and better availability of the drug.
- The size of the particle is reduced through various stages like pounding, sieving, filtering through white cloth *(vasthirakayam)*
- Only if the size of the particle is reduced to micro particles, the drug is easily absorbable in the digestive system.
- The above processes reduced the size of particle so that the *chooranam* passes through the sieve no 88.

PHYSICO CHEMICAL ANALYSIS

Table no 10: Results of Physico chemical analysis of *Eladhi chooranam*

S.No	Parameter	Result
1.	P^H	4.24
2.	Solubility	Positive
	Distilled water	Soluble
	Benzene	Soluble
3.	Loss on drying	9.61%
4.	Total ash value	5.47%
5.	Acid insoluble Ash (%)	1.5%
6.	Water soluble ash (%)	0.99%
7.	Water soluble extraction	21.6%
8.	Alcohol soluble extraction	16%

Discussion:

The physico chemical analysis of the drug result reveals the pH, Solubility, Loss of drying, Total ash value, Water soluble ash and Acid insoluble ash.

Solubility:

- Solubility is one of the important parameters to achieve desired concentration of drug in systemic circulation for desired (anticipated) pharmacological response.
- Oral ingestion is the most convenient and commonly employed route of drug administration; oral bio-availability depends on solubility**.**
- *EC* is soluble in major solvents and sparingly soluble in some solvents, well soluble in Distilled water, Benzene. It improves that its efficiency of solubility and increases the bio- availability of the test drug [77].

pH:

- The pH level plays a role in enzyme activity by maintaining the internal environment, thus it exhibits important role in regulating homeostasis.
- *Eladhi chooranam* shows weak acidic pH 4.24.
- Weak acid is more lipids soluble in an acidic solution, and more water soluble in alkaline solution.
- Whenever a weak acid drug is given, most of the drug in the stomach is in un-ionized form, which forms through the gastric mucosa. Weakly acidic drugs are more readily absorbed from an acid medium (stomach) than are weakly basic drugs.
- It is also important factor for drug absorption. This enhances the bio-availability. [78]

Loss on drying:

Loss on drying (LOD) gives the total amount of volatile content and moisture (water) present in the drug.

- The stability of a drug and its shelf-life are dependent on moisture content.
- Moisture increase can adversely affect the active ingredient.
- Low moisture content- drug could get maximum stability and better shelf life.
- Since the *EC* has low loss on drying, the moisture content is 9.61% which is suitable for medicine preparation.

Ash:

- Ash values are helpful in determining the quality and purity of the drugs
- The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration [79].
- The total ash value of *Eladhi chooranam* is 5.47%, which determines the presence of inorganic content.

Acid insoluble ash:

- The total ash is the residue remaining after incineration. The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid.
- Lower the acid insoluble value better will be the drug quality. The drug ensures a low value of acid insoluble ash indicating that the preparation did not contain any sand, dust and stones.
- The Acid insoluble ash of *Eladhi chooranam* is 1.5% which ensures the trail drug does not contain any sand, dust and stones.

Water soluble ash:

- Decreased water soluble ash value indicates easy facilitation of diffusion and osmosis mechanisms.
- The Water-soluble ash of *Eladhi chooranam* is 0.99% indication increase the facilitate of diffusion and osmosis.

Water-soluble and Alcohol-soluble extraction

- Water-soluble extractive values of ingredients and formulation of ***Eladhi chooranam*** are depicted in table which shows 21.6%.
- Higher water-soluble extractive value implies that water is a better solvent of extraction for the formulation than ethanol.
- Alcohol-soluble extractive values of ingredients and formulation of ***Eladhi chooranam*** are depicted in table which shows 16%.
- The results of Alcoholic and Water-soluble extracts of the formulation show that alkaloids of the formulations are more soluble in water than alcohol and a higher water-soluble extractive value of the formulation depicts that water is a better solvent of extraction for the formulation than alcohol.

Table no 11: Results of phytochemical analysis of *Eladhi chooranam*

S.no	Phytochemicals	Test name	Result
1.	Alkaloids	Dragendroff's Test	+ve
2.	Glycoside	Keller-Killiani Test	+ve
3.	Saponin	Froth Test	+ve
4.	Phenols	Ferric chloride Test	+ve
5.	Flavanoids	Alkaline Reagent test	+ve
6.	Proteins	Xanthoproteins Test	+ve
7.	Diterpines	Copper Acetate Test	+ve
8.	Gum and Mucilage	Extract +Alcohol	+ve
9.	Quinones	NAOH+Extract	+ve

+ve/-ve present or absent if component tested

Fig No 15: Preliminary Phytochemical Analysis of Eladhi Chooranam

Discussion

Phytochemicals are natural bioactive compounds, found in plants and fibres, which act as a defense system against diseases and more accurately protect against diseases. The phytochemical analysis reveals that the presence of Alkaloids, Glycosides, Saponin, Phenol, flavonoids, Protein, and Diterpines, Gum and Mucilage

Alkaloids:

- Alkaloids possess Vasodilators and anti-arrhythmic effects
- Alkaloids are the active principles producing many essential effects in protecting the body [80].

Glycosides

- Glycosides are Anti-oxidant activity in nature thus it plays major role in treating cardiac diseases [81].

Saponin:

- Saponins reduce an emulsification of fat molecules. Saponin binds with bile salt and cholesterol in the intestinal tract. Bile salts form small micelles with cholesterol facilitating its absorption. Saponin cause a reduction of blood cholesterol by preventing its re-absorption.
- It has a property of Anti-oxidant and reduced the risk of heart diseases [82].

Phenols:

- They possess rich Anti-Oxidant property and protect body from oxidative stress [83]

Diterpene:

- Diterpene has the property of exhibiting of vasorelaxant action and inhibiting of vasocontraction
- So, it is helpful in reduction of blood pressure [84].

Protein

- They help in repairing cells and important for growth [85].
- Increasing protein intake may actually help to lowering systolic blood pressure by more than 2mmHg in comparison with intake of carbohydrates.
- Both animal and plant proteins lowered BP and led to statistically significant reductions in HBP risk [86].
- A synergistic effect of all these alkaloids, glycosides, phenols, triterpenes, flavanoids, quinones increases the potency of the drug against hypertension.

Flavonoids

- It is the most important group of polyphenol compounds in plants.
- They improve the endothelial and capillary function
- Reduces the risk of atherosclerosis.
- They help in strengthening and protect the inner lining of the blood vessels
- Flavonoids are a group of plant metabolites which provide health benefits through cell signaling pathways and antioxidant effects.
- Flavonoids can exert their Anti-Oxidant activity by scavenging the free radicals, by chelating metal ions or by inhibiting enzymatic systems which are responsible for free radical generation [87].

Tannin:

- Tannins contains anti-oxidant effect which producing many essential effects in protecting the body.
- Tannin impacts on blood pressure and urinary parameters.
- Tannin contains ACE inhibitory effect [88].

Quinones

- Quinone possesses anti-oxidant activity.
- Quinone activation reduces blood pressure.
- Prevent cardiovascular diseases [89].

Table no 12: High Performance Liquid Chromatography (HPLC) analysis of *Eladhi chooranam:*

HPLC analyses were done. HPLC analysis performed with *Eladhi chooranam Chooranam* revealed the presence of following compounds:

S. NO	PARAMETERS	METHOD	UNITS	RESULTS
1.	Total Polyphenol as gallic acid Equivalent	Indian Pharmacopoeia 2014	mg/100g	0.52
2.	Total Flavonoids as Quercetin Equivalent	TNTH/STP/FOOD/110	mg/100g	6.20
3.	Total Alkaloids	TNTH/STP/FOOD /426	mg/100g	3.26
4.	Total Tannin as Tannic Acid Equivalent	AOAC 20th Edn.2012, 955.35	mg/100g	2.10

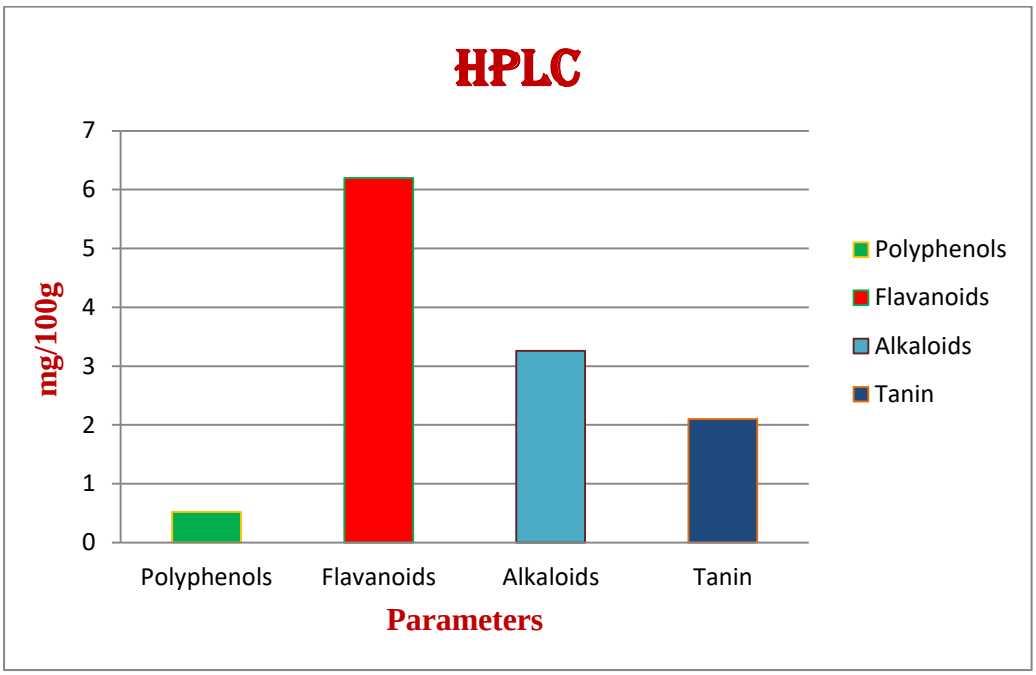


Chart no:1 Results of HPLC analysis

Interpretation:

Polyphenols:

- Polyphenols are protection and improving endothelial function with vascular relaxation and improves vascular health, thereby significantly reducing the risk of hypertension and CVD.
- Polyphenols can stimulate the formation of vasoprotective factors such as nitric acid (NO) and endothelium derived hyperpolarizing factor to promote vasodilation. Polyphenols inhibits platelet aggregation in humans.
- Polyphenols also improve vascular smooth muscle function, by reducing the excessive vascular oxidative stress of pathological blood vessels associated with many cardiovascular risk factors.
- Polyphenols are the members of very large family of plant derived compound which had the anti-lipogenic effect. This is mainly due to the reduce fatty acid and triglycerol synthesis, increased in fatty acid oxidation and reduced oxidative stress and inflammation [90].

Flavanoids:

- Flavonoids are able to modulate blood pressure by restoring endothelial function, either directly or by affecting nitric acid levels.
- Flavanoids comprise large group of secondary metabolites occurring widely throughout the plant kingdom.
- Biological action of flavonoids, including anti-oxidant, anti aggregant and vasodilator affect.
- Flavanoids can also reduce caloric intake and decrease body weight and fat deposition in visceral tissues.
- Flavanoids are the unique antioxidant. It also corrects dislipidemia and blood pressure [91].

Tannin:

- Tannins contains anti-oxidant effect which producing many essential effects in protecting the body.
- Tannin contains ACE inhibitory effect [92].

BIOCHEMICAL ANALYSIS:

Table no 13: Results of basic radical studies of *Eladhi chooranam*

S.No	Parameter	Observation	Result
1.	Test for Potassium	Formation of yellow colour precipitate	Positive
2.	Test for Calcium	Formation of white colour precipitate	Positive
3.	Test for Magnesium	Formation of white colour precipitate	Positive
4.	Test for Ammonium	Appearance of brown colour	Positive
5.	Test for Sodium	Appearance of intense yellow colour	Positive
6.	Test for Zinc	Formation of white colour precipitate	Positive

Discussion

The results of basic radical test show that the presence of Potassium (K^+), Calcium (Ca), Magnesium (Mg), Sodium (Na) and Zinc (Zn).

Potassium

- Potassium is important for muscle function especially relaxing the wall of blood vessels.
- This lowers the blood pressure and protects against muscle cramping.
- This protects against an irregular heartbeat [93].

Calcium:

- Presence of calcium improves the physical strength of skeletal tissue. calcium ions are necessary for muscle contraction and transmission of nerve impulse.
- Calcium is important for healthy blood pressure because it helps in vasoconstriction and vasodilatation of blood vessels [94].

Magnesium

- Magnesium also helps in regulation of blood pressure and relaxing the blood vessels.
- Magnesium act as a natural calcium channel blocker, increases nitric oxide, improves endothelial dysfunction and induces vasodilation [95].

Ammonium Screenshot

- Ammonium predicts clinical outcomes in Hypertensive kidney diseases
- Ammonia may contribute to the vasodilatation and increase in cerebral blood flow.

Sodium

- Sodium also important for regulation of blood pressure [96]

Zinc

- Zinc have an Anti-oxidant property
- Helps to protect cells in the body from damage caused by free radicals[97]

Table no 14: Results of acid radical studies of *Eladhi chooranam*

S.NO	Parameter	Observation	Result
1.	Test for Sulphate	Appearance of white precipitate	Positive
2.	Test for Chloride	Formation of white precipitate	Positive

Discussion:

The acidic radicals test shows the presence of Chloride and Nitrate.

Sulphate

- which is primarily used for its anticonvulsive effects in treating hypertensive disorders of pregnancy [98].

Chloride

- They help in maintenance of proper blood volume, blood pressure, pH of blood and also help in balance between ECF and ICF of cells [99].

ANTI-MICROBIAL ACTIVITY OF *ELADHI CHOORANAM*

GRAM NEGATIVE BACTERIA

Table no 15: Organism: *E.coli*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Eladhi Chooranam (EC)	Streptomycin (100µg)	26
	250	18
	500	21
	1000	25

14mm – Low sensitive, 15mm – Moderate, 16mm – Highly sensitive

Table no 16: Organism: *Klebsiella pneumoniae*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Eladhi Choornam (EC)	Streptomycin (100µg)	25
	250	18
	500	20
	1000	23

14mm – Low sensitive, 15mm – Moderate, 16mm – Highly sensitive

Table no 17: Organism: *Pseudomonas aeroginosa*

Sample	**Concentration (µg/mL)**	**Zone of inhibition (mm)**
Eladhi Choornam (EC)	Streptomycin (100µg)	30
	250	20
	500	25
	1000	29

14mm – Low sensitive, 15mm – Moderate, 16mm – Highly sensitive

GRAM POSITIVE BACTERIA

Table no 18: Organism: *Staphylococcus aureus*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Eladhi Choornam (EC)	Streptomycin (100µg)	26
	250	19
	500	23
	1000	27

14mm – Low sensitive, 15mm – Moderate, 16mm – Highly sensitive

Table no 19: Organism: *Aspergillus niger*

Sample	Concentration (µg/mL)	Zone of inhibition (mm)
Eladhi Choornam (EC)	Clotrimazole(100µg)	37
	250	21
	500	25
	1000	32

14mm – Low sensitive, 15mm – Moderate, 16mm – Highly sensitive

Inference:

1. *Escherchia coli* - Highly sensitive in 250(µg/mL)
2. *Klebsiella pneumoniae* - Highly sensitive in 250 (µg/mL)
3. *Pseudomonas aeruginosa* - Highly sensitive in 250(µg/mL)
4. *Staphylococcus aureus* - Highly sensitive in 250 (µg/mL)
5. *Aspergillus niger* - Highly sensitive in 250 (µg/mL)

Discussion:

The development of resistance against the presently available antibiotics arises the necessity of rediscovery of new anti-bacterial and anti-fungal agents in traditional systems of medicine. Different dosages of test drug against the microbes in antimicrobial activity of *EC* was compared with Standard drug Streptomycin and Clotrimazole (100µg)/ml disc for the following pathogens, they are *Escherchia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus,*

Aspergillus niger. The results represents *EC* potentially inhibit the growth of all above organism in 250µl, 500µl and 1000µl / disc. 14 mm – Low sensitive, 15 mm – Moderate, above 16 mm – Highly sensitive. The findings reveal that the Siddha drug *EC* have anti-microbial potency against bacterial and fungal pathogens which is used in the treatment of diseases.

INSTRUMENTAL ANALYSIS

FT-IR (Fourier Transform Infra-Red spectroscopy)

Fourier Transform Infra-Red Spectroscopy (FTIR) analysis results in absorption spectra provide information about the functional group and molecular structure of a material. The results of Table no:20 and Fig no:7 shows the presence of functional group and inorganic compounds of *Eladhi chooranam*

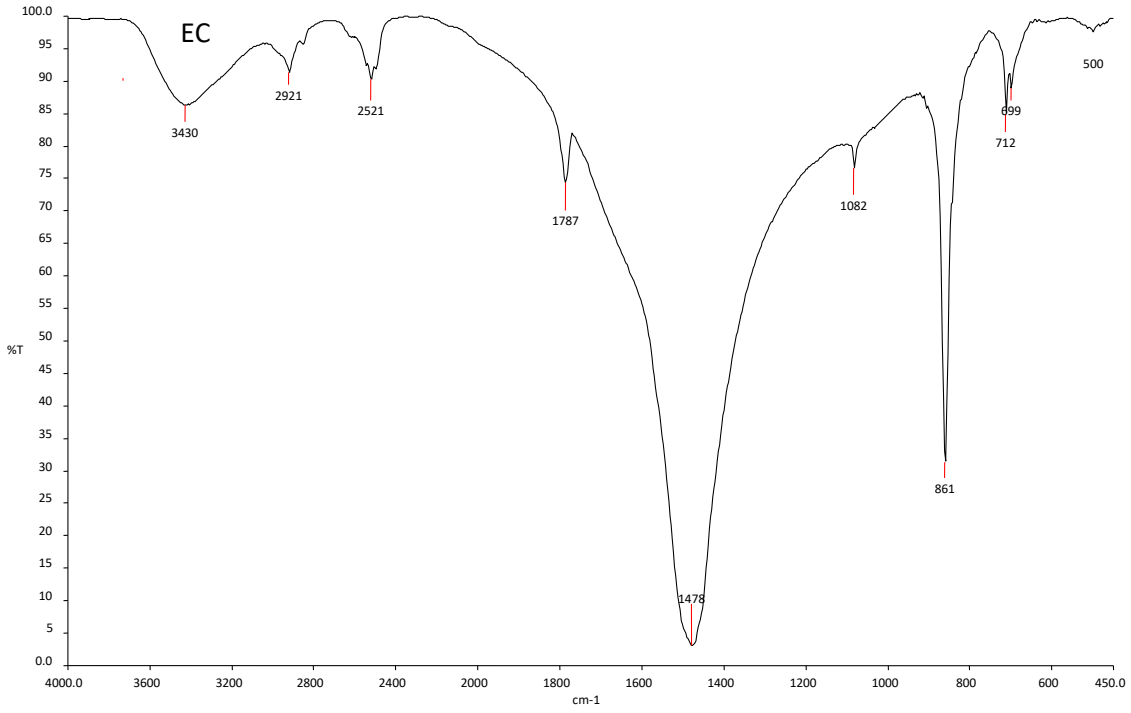


Fig no 7: FT-IR Spectrum analysis

Table no 20: FTIR-INTERPRETATION of *Eladhi chooranam*

Absorption peak cm^{-1}	**Stretch**	**Functional group**
3430	O-H stretch, free hydroxyl	Alcohols, Phenols
2921	C-H Stretch	Alkene
2521	H-C=O:C-H Stretch	Aldehydes
1787	C=O Stretch	Carbonyls (General)
1478	N-O asymmetric Stretch	Nitro compounds
1082	C-O Stretch	Alcohols, Carboxylic acids, Esters, Ethers
861	=C-H bend	Alkenes
712	N-H wag	1^0, 2^0 amines
699	C-H "oop"	Aromatics

DISCUSSION

FTIR instrumental analysis was done. The test drug was identified to have 9 peaks. They are the functional groups present in the trial drug "*Eladhi Chooranam".* The above table shows the presence of alcohol, Phenols, Alkenes, Aldehyde, Carbonyls, Nitro compounds, Carboxylic acids, Esters, Ethers, Amine, Aromatic groups which represent the peak value.

Phenols:

- It has possessed high Anti-Oxidant property which enhances the drug effect against the disease [100].

Alcohols:

- The world Hypertension league speculated that the relatively greater effect alcohol on systolic blood pressure compared with diastolic blood pressure may indicate an imbalance between central nervous system factors influencing cardiac output and the peripheral vascular effects of alcohol.
- There is increasing evidence that alcohol initiates central as well as peripheral reactions which in a synergistic manner have hypertensive action [101].

Aldehydes:

- The clear benefit and need for aldehyde-conjugating therapies exists for cardiovascular disease [102].

Amines:

- It enhances the drug effect against the disease.
- Amines groups act as neurotransmitters. Amines are a class of compounds derived from ammonia by replacement of one or more effective antagonists of SSTR5 (Somatostatin receptor 5) and are used for treatment, control and prevention of disorders such as lipid disorders and obesity [103].

Nitro groups:

Nitro groups containing drugs act directly on the vascular smooth muscle to cause relaxation and reduce the blood pressure [104]

SEM: (SCANNING ELECTRON MICROSCOPE)

The particle size and the chemical elements were assessed by Scanning Electron Microscope. SEM is one of the most widely used instruments in research side. The SEM picture of *Eladhi Chooranam* is shown below.

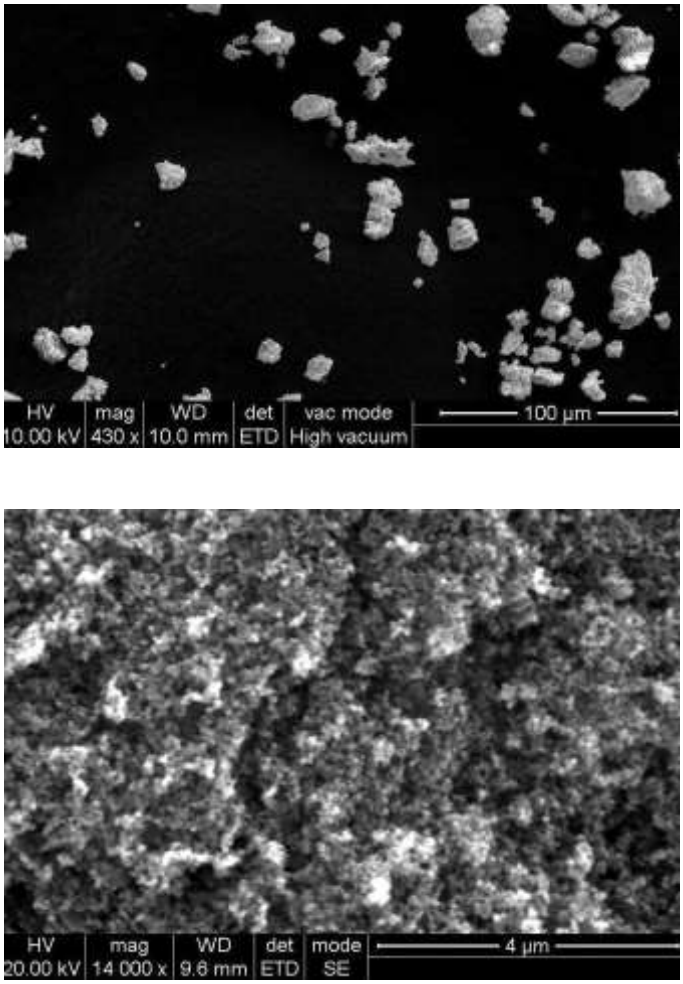


Fig no 8: SEM analyses

The particle morphology can be identified through these SEM images of Siddha medicine *Eladhi chooranam*. The particles are not spherical in shape. The size of the particles was approximately identified between 5-1micron.

Discussion for SEM

- Micro particles are defined as particulate dispersion or solid particles with a size in the range of 100-1000 nm in diameter.
- Size and surface of micro particles can be easily manipulated to achieve both passive and active drug targeting.
- They control and sustain the release of drug during the transportation and at the site of localization, alter the drug distribution in the body and subsequent clearance of the

drug so as to achieve increased drug therapeutic efficacy there by it increases the bio-availability of the drug and reduced the side effects.

Hence, *Eladhi chooranam* which is prepared biologically contains micro particles to enhance the pharmacological action in the target site [105].

ICP-OES (Inductively Coupled Plasma Optic Emission Spectrometry):

The drug sample *Eladhi Chooranam* was analysed by the Inductively Coupled Plasma Optic Emission Spectroscopy (ICP-OES) to detect the trace elements and other elements quantitatively. The result of (ICP-OES) is given on the

Table No 21: Results of ICP-OES

S.No	Elements Symbol	Wavelength (nm)	Concentration
1.	Aluminium (Al)	396.152	BDL
2.	Arsenic (As)	188.979	BDL
3.	Calcium (Ca)	315.807	13.105 mg/L
4.	Cadmium (Cd)	228.802	BDL
5.	Copper (Cu)	327.393	BDL
6.	Iron (Fe)	238.204	01.004 mg/L
7.	Mercury (Hg)	253.652	BDL
8.	Magnesium (Mg)	285.213	01.274 mg/L
9.	Sodium (Na)	589.592	34.801 mg/L
10.	Nickel (Ni)	231.604	BDL
11.	Lead (Pb)	220.353	BDL
12.	Phosphorous (P)	213.617	98.107 mg/L

Discussion:

- The above results indicate that the trial drug is extremely safe as it contains heavy metals within specified limits.
- In *Eladhi Chooranam*, the heavy metals like As, Cd, Hg, Pb and were below detectable level. This reveals the safety of the drug.
- In addition *Eladhi chooranam* has the presence of Calcium (Ca) 13.105 mg/L, Iron (Fe) 01.004 mg/L, Magnesium (Mg) 01.274 mg/L, Sodium (Na) 34.801 mg/L, Phosphorous (P) 98.107 mg/L are detected.

XRD (X-RAY DIFFRACTION METHOD)

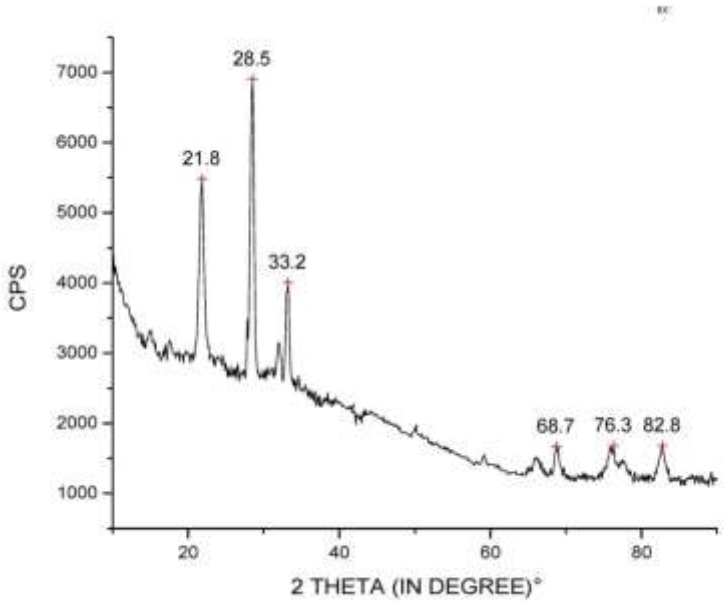


Fig no 9: XRD analysis

Discussion

The crystalline structure, the size and shape of the particles are highly dependent on the route of synthesis and highlight the efficacy of the drug. The nano particles may enhance bio absorption of the drug.

XRD pattern of *Eladhi chooranam* shows the good crystallinity after calcinations process. The major diffraction peaks are identified after XRD analysis *EC* concluded that Nano crystalline range (21-33nm) is association with organic molecules probably plays an important role in making it biocompatible and nontoxic at therapeutic doses. Other elements present in *EC* act as additional supplement and possibly helps in increase the efficacy of the formulation [106].

TOXICITY STUDY RESULT

ACUTE ORAL TOXICITY IN RATS – OECD 423

Wistar albino rat was treated with the test drug *Eladhi Chooranam* of single dose of 2000mg/kg in 2% CMC as suspention. This study was conducted as per the OECD guidelines. The result of acute toxicity of *Eladhi Chooranam* has been tabulated below.

Dose finding experiment and its behavioural Signs of Toxicity for *Eladhi Chooranam*

Table no 22: Observation done

SL	Group CONTROL	Observation	SL	Group TEST GROUP	Observation
1	Body weight	Normal	1	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	No significant color change

8	Piloerection	Normal	8	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Normal
10	Sensitivity response	Normal	10	Sensitivity response	Normal
11	Locomotion	Normal	11	Locomotion	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal
13	Rearing	Mild	13	Rearing	Mild
14	Urination	Normal	14	Urination	Normal

Table no 23: observational study results of *Eladhi chooranam*

No	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	**Control**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-
2.	**2000mg/ kg**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15. Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality.

(+ Present, - Absent)

Table no 24: Body weight (g) changes of rats exposed to *Eladhi Chooranam*

DOSE	**DAYS**		
	1	7	14
CONTROL	270.1±65.70	272.7±09.71	270.06±2.10
High DOSE	260.3±4.44	261.4±7.12	260.2±6.05
P value (P*)	NS	NS	NS

*N.S- Not Significant, **(p >0.01), *(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table no 25: Water intake (ml/day) of Wistar albino rats group exposed to *Eladhi chooranam*

DOSE	**DAYS**		
	1	6	14
CONTROL	60±1.62	62±1.10	69.1±1.04
High DOSE	69.5±1.04	69.5±2.07	71.8±2.04
P value (P*)	NS	NS	NS

*N.S- Not Significant, **(p >0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table no 26: Food intake (gm/day) of Wistar albino rats group exposed to *Eladhi chooranam*:

DOSE	DAYS		
	1	7	14
CONTROL	62.4±1.54	62.2±1.62	62.7±4.06
High DOSE	64.0±2.24	64.4±2.10	64.6±2.70
P value (P*)	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Discussion:

- In the acute toxicity study, the rats were treated with different concentration of ***Eladhi chooranam*** from the range of 5mg/kg to 2000mg/kg.
- The test groups compared to the controls when observed during14 days of the acute toxicity experimental period. This dose level of *EC* did not produce signs of toxicity, behavioural changes, Body weight and mortality.
- No significant alterations were observed in food and water intake.
- These results showed that a single oral dose of the extract showed no mortality of these rats even under higher dosage levels indicating the high margin of safety of this extract.
- In acute toxicity test, based on OECD 423 the trial drug *Eladhi chooranam* was found to be nontoxic at the dose level of 2000mg/kg body weight.

RESULTS OF SUB-ACUTE ORAL TOXICITY 28 DAYS REPEATED DOSE STUDY IN RATS

Table no 27: Body weight (g) changes of rats exposed to *Eladhi Chooranam*

DOSE	DAYS				
	1	7	14	21	28
CONTROL	180.6±3.62	181.4±4.14	183.7±9.61	184.6±3.03	185.7±1.31
LOW DOSE	183.2±1.14	182.7±3.64	180.4±8.32	180.1±4.66	178.4±3.76
MID DOSE	186.6±1.64	184.3±2.74	182.4±8.32	181.1±3.16*	177.7±3.82**
HIGH DOSE	184.4±6.74	182.6±2.12	178.6±2.36*	172.2±4.78	174.12±2.39**
P value (P*)	NS	NS	NS	NS	NS

*NS- Not Significant, **(p > 0.01),*(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table no 28: Water intake (ml/day) of Wistar albino rats group exposed to *Eladhi chooranam*

DOSE	DAYS				
	1	6	14	21	28
CONTROL	61.5±8.95	61.5±6.23	62.7±6.23	62.6±8.196	62.9±3.96
LOW DOSE	68.5±3.12	68.4±4.12	68.8±3.24	68.2±1.28	68.7±4.23
MID DOSE	69.7±1.23	69.3±2.11**	69.1±1.13	69.4±1.21**	69.4±1.14
HIGH DOSE	63.1±1.12	63.2±2.23**	63.4±1.23	63.2±2.33*	63.4±1.25**
P value (P*)	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p > 0.01), *(p >0.05), n = 12 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table no 28: Food intake (gm/day) of Wistar albino rats group exposed to *Eladhi chooranam*

DOSE	DAYS				
	2	7	23	22	28
CONTROL	37.12±5.37	38.5±3.22	39.5±3.37	39.5±3.37	40±3.12
LOW DOSE	39.7±2.98	39.3±2.32	39.1±7.28*	40.4±2.92	41.6±1.62*
MID DOSE	40.2±1.25	40.2±1.20	41.2±2.15	41.4±1.28	41.7±2.44**
HIGH DOSE	41.3±1.24	41.6±1.24	42.7±2.66*	42.6±1.20*	42.1±3.12**
P value (P*)	NS	NS	NS	NS	NS

*N.S- Not Significant, **(p >0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table No 30: Haematological parameters of Wister albino rats group exposed to *Eladhi chooranam*

Category	Control	Low dose	Mid dose	High dose	P value (p)*
Haemoglobin(g/dl)	13.8±0.88	13.90±1.16	15.14±0.66	16.28±1.16	N.S
Total WBC ($\times10^3$ l)	11.91±0.59	11.85±1.23	11.08±1.21	11.11±2.27	N.S
Neutrophils (%)	33.65±0.06	33.3±1.24	32.11±2.16	33.20±1.10	N.S
Lymphocyte (%)	70.24±1.48	70.02±1.12	70.20±1.16	70.10±1.26	N.S
Monocyte (%)	0.86±0.07	0.58±0.19	0.72±0.13	0.71±0.60	N.S
Eosinohil(%)	0.54±0.09	0.54±0.12	0.62±0.16	0.72±0.04	N.S
Platelets cells10^3/µl	687.17±8.76	678.71±9.16	675.18±2.20	672.16±3.74	N.S
Total RBC 10^6/µl	7.99±0.12	7.79±1.57	7.62±0.19	7.05±0.12	N.S
PCV%	37.79±0.6	37.35±1.23	37.98±1.18	36.82±2.14	N.S
MCHC g/dL	33.6±2.23	33.29±1.19	33.18±1.12	34.03±1.14	N.S
MCV fL(µm^3)	49.07±3.64	49.28±8.12	49.20±1.24	49.2±1.94	N.S

*N.S- Not Significant, **(p >0.01), *(p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table no 31: Biochemical Parameters of Wistar albino rats group exposed to *Eladhi chooranam*

BIOCHEMICAL PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
GLUCOSE (R) (mg/dl)	74.45±13.4	74.16±1.24	74.26±1.22	75.12±9.60	N.S
T.CHOLOSTEROL(mg/dl)	115.26±1.83	113.45±1.13	108.42±1.78	99.22±1.93	N.S
TRIGLY(mg/dl)	46.35±1.48	44.22±1.28	42.58±1.80	39.66±1.13*	N.S
LDL	73.8±2.43	70.24±3.14	68.14±1.24	65.64±4.12	NS
VLDL	15.2±2.44	15.82±1.14	15.44±2.14	15.24±4.16	NS
HDL	26.66±6.88	26.16±1.24	26.68±2.16	26.78±1.12	NS
Albumin(g/dL)	3.3±0.17	3.23±0.22	2.48±2.02	3.14±3.16	NS

*NS- Not Significant, **(p >0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test*

Table no 32:Renal function test of of Wistar albino rats group exposed to *Eladhi chooranam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
UREA (mg/dl)	13.35±0.99	14.81±1.26	16.26±1.18	21.28±3.12	N.S
CREATININE(mg/dl)	0.58±0.08	0.48±0.06	0.72±0.14	0.74±0.12	N.S
BUN(mg/dL)	15.12±0.10	15.12±0.28	16.28±0.14	16.90±1.22	N.S
URIC ACID(mg/dl)	5.37±0.35	5.11±0.43	6.72±2.15	6.28±0.14	NS

*NS- Not Significant, **(p >0.01), * (p >0.05) , n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Table 33: Liver Function Test of of Wistar albino rats group exposed to *Eladhi chooranam*

PARAMETERS	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE	P Value (p)*
T BILIRUBIN(mg/dl).	0.05±0.07	0.05±0.16	0.06±0.18	0.05±0.15	N.S
SGOT/AST(U/L)	114.95±1.39	115.15±2.11	116.21±1.23	116.55±1.23	N.S
SGPT/ALT(U/L)	71.23±1.28	72.91±1.59	72.34±2.18	71.32±1.28	N.S
ALP(U/L)	146.25±8.77	144.2±6.27	149.16±4.17*	153.3±4.25*	NS
T.PROTEIN(g/dL)	6.32±0.38	6.48±1.34	6.52±0.23	6.53±1.26	N.S

*NS- Not Significant, **(p > 0.01), * (p >0.05), n = 10 values are mean ± S.D (One way ANOVA followed by Dunnett's test)*

Results:

Observations

Overall observations were similar in both sex rats.

Clinical signs of toxicity

No clinical signs of toxicity were observed.

Mortality

No mortality was observed after 28 days repeated dose administration of *EC*. All animals survived to study termination period.

Body weight

The result of the body weight of rats exposed to control and the trial drug of different dose groups exhibited overall mild weight loss throughout the dosing period of 28 days. The quantity of food taken by the animals from different dose groups and the control is comparably normal.

Food and water consumption

No effect of treatment was noted.

Physiological activities

No changes in the general behavior

Blood analysis

a. **Hematology**

   No treatment related effects were observed.

b. **Biological parameters**

   No treatment related effects were observed.

c. **Histological examination**

   Histological examination of organs did not show any pathological changes.

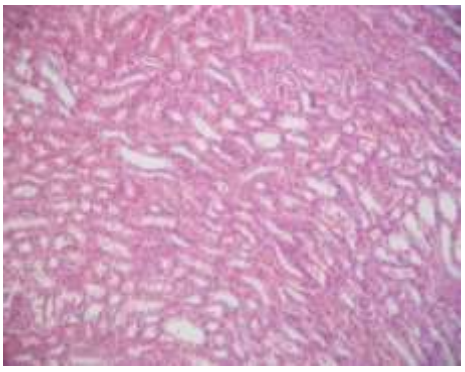
Repeated Oral Toxicity Discussion:

- The repeated 28 days' oral toxicity studies of *EC* showed that the drug did not produce any toxicity signs in wistar albino rats. Daily administrations of EC at different doses 20mg/kg, 200mg/kg, 400mg/kg for 28 days were tolerated by the rats without any mortality and morbidity indicates the drug tolerance.
- No physical changes were observed throughout the dosing period.
- No significant changes were observed in the values of different parameters studied when compared with controls and the values obtained were within normal biological and laboratory limits.
- No significant changes in Red blood cells (RBC) white blood cell (WBC), packed cell volume (PCV) in all the treated groups as compared to respective control groups.
- Hence the Poly herbal formulation of *EC* can be considered to be safe drug for prolonged duration use as revealed by toxicological studies.

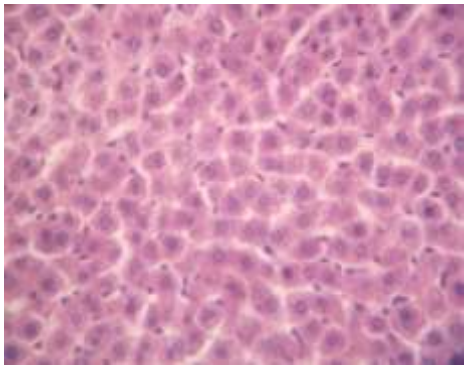
HISTOPATHOLOGY

Control group

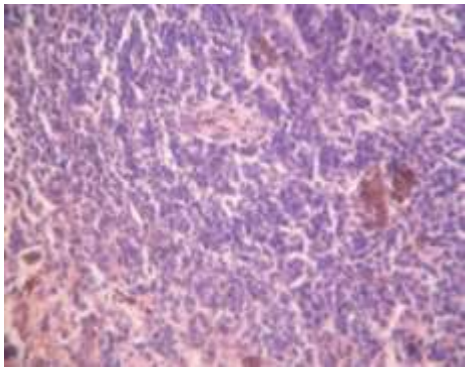
Kidney



Liver

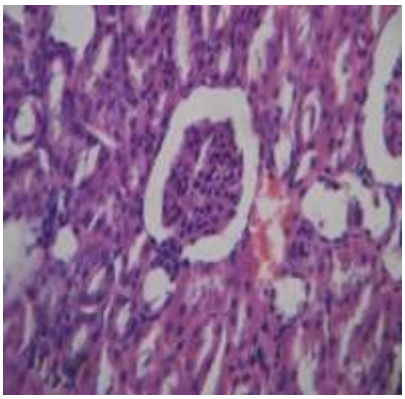


Spleen

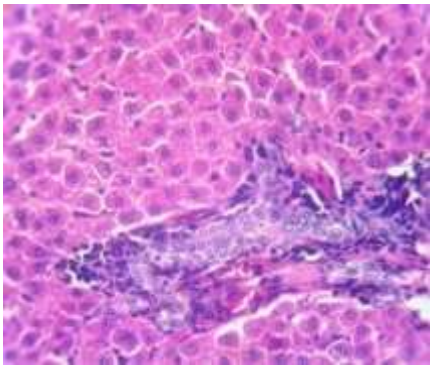


***EC* 400 mg**

Kidney



Liver



Spleen

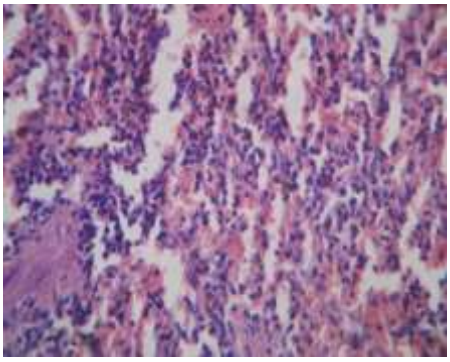


Fig no: 10

Discussion

The above slides show the histopathology studies of sub-acute toxicity. There is no toxicological abnormality seen in the vital organs after administration of the test drug *Eladhi chooranam*. Thus the safety of the drug is revealed, so that it can be administered for long time without any side effects

PHARMACOLOGICAL STUDY

ANTI-HYPERTENSIVE ACTIVITY

SHR (9 weeks old) and age-matched Wistar rats male weighing 250±20g, Rats were kept in a room temperature controlled room (25 ° C), with 12 h dark and 12 h artificial illumination daily (7:00— 19:00). Food and water were available ad libitum. Systolic blood pressure (SBP) and heart rate measurement of SH rats was carried out using tail-cuff method plethysmography (LE 5001 Pressure Meter).

GROUPING

The animals were divided into following groups:

Table No 34: Grouping of animals

Group I	Control untreated group which received normal saline.
Group II	Received Verapamil 12.5 mg/kg b.w
Group III	ELADHI CHOORANAM 200mg/kg b.w
Group IV	ELADHI CHOORANAM 400mg/kg b.w

The drug *Eladhi chooranam* was administered orally and once daily for 4 weeks.

Table no 35: Effect on Systolic Blood Pressure (SBP) of *Eladhi Chooranam* on various treatment groups on SH-rats

S:NO	Treatment group	**SBP** Initial	7th day	14th day	21st day	28th day
1.	Control	220.3±4.15	210.4±6.46	208.2±2.86	202.6±2.64	200.3±8.65
2.	*EC* 200	210.4±6.86	196.8±6.82	170.2±4.62	161.8±8.42	152.4±2.14 **
3.	*EC* 400	190.8±6.24	182.2±6.24	165.2±4.82	150.8±8.26	126.2±3.12 ***
4.	Verapamil hydrochloride 12.5mg/kgb.w	182.6±4.28	170.2±2.46	158.6±4.86	142.4±6.40	110.2±2.08 ***

Values represent mean ± SEM of 6 experiments. P< 0.05; ** P< 0.01; *** P < 0.001, treatment versus control group*

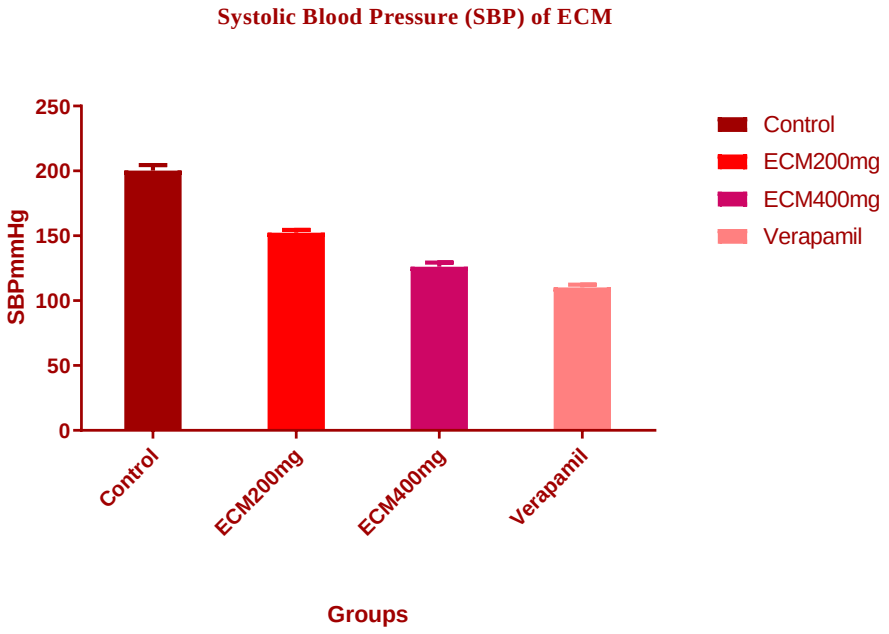


Chart no.1. Effect of Systolic Blood Pressure (SBP) of *EC* on various treatment groups on SH-rats at 28th day

Table no 36: Effect on Heart rate (HR) of *Eladhi chooranam* various treatment groups on SH-rats

S:NO	Treatment group	**HR beats/minute** Initial	7th day	14th day	21st day	28th day
1.	Control	498.2±5.81	496.6±2.86	493.7±4.01	491.8±8.10	490.2±6.43
2.	*EC* 200	490.6±2.84	480.8±4.21	462.2±4.01	446.2±4.82	436.4±1.02 **
3.	*EC* 400	482.6±4.81	455.21±8.24	421.8±4.46	390.8±4.81	367.2±2.21 ***
4.	Verapamil hydrochloride 12.5mg/kgb.w	470.6±6.21	438.6±8.24	400.8±8.62	364.68±2.1	320.1±1.14 ***

Values represent mean ± SEM of 6 experiments. P< 0.05; ** P< 0.01; *** P < 0.001, treatment versus control group*

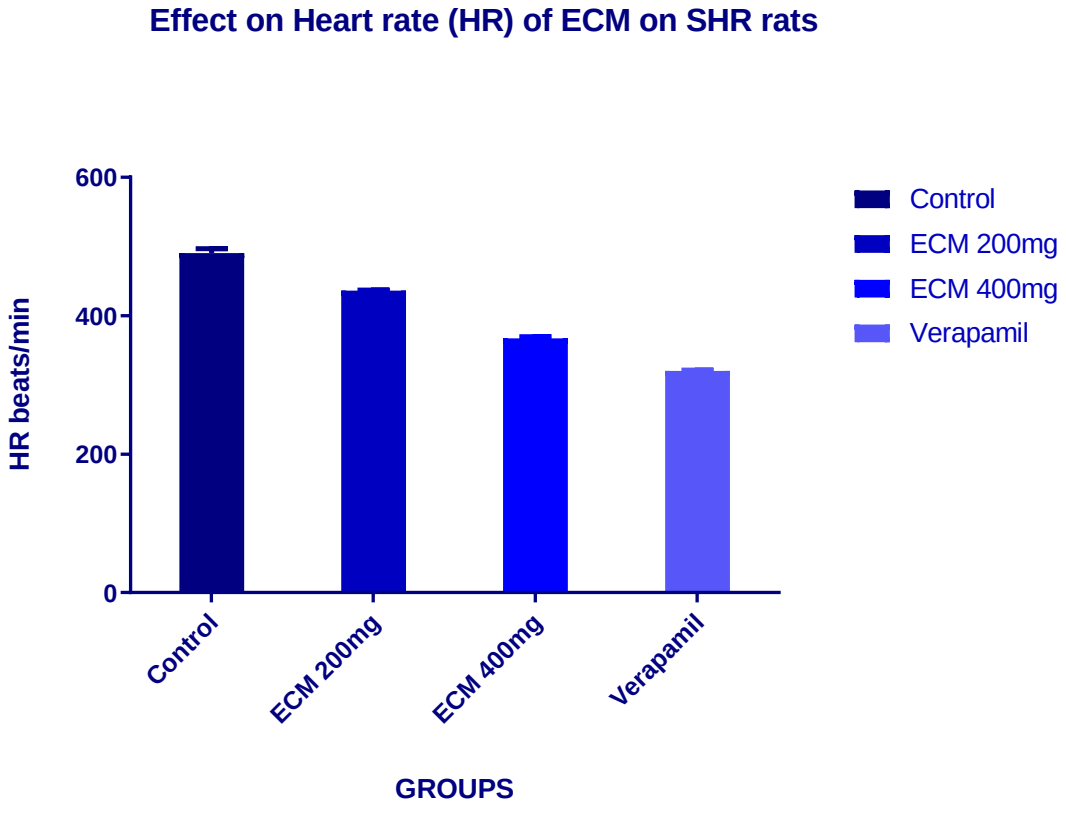


Chart no.2. Effect on Heart rate (HR) of *Eladhi Chooranam on* various treatment groups on SH-rats at 28th day

Discussion:

- The systolic blood pressure and heart rate were recorded in the conscious animals in non-invasive tail cuff method.
- The results reveals that the *EC* exhibits antihypertensive effect in the form of significant lower in systolic blood pressure and heart rate after continued administration for 7 days.
- Heart rate was also decreased significantly in comparison to control
- The reduction in systolic blood pressure was measured and tabulated. The readings were compared with control group
- The systolic blood pressure on 7^{th} day in group III treated with *EC* 400m/kg body weight showed moderate reduction in Systolic blood pressure compared with 7^{th} day of control
- But the reduction of Systolic blood pressure measured on 21^{st} day of *EC* 400mg /kg body weight treated group showed significant reduction of Systolic blood pressure compared with 21^{st} day of control group persistence highly significant antihypertensive effect was noticed even after cessation of dosing 7 days earlier. This suggests absence of rebound phenomenon after withdrawal of the test drug *EC* which an advantage in the therapy of hypertension.
- The Siddha Polyherbal formulation *EC*, according to their traditional uses and phytochemical constituents based on their therapeutic value leads to discovery of newer and safer alternative drug and herbal medicines having a protective role in cardiovascular diseases.

DIURETIC ACTIVITY

Lipschitz et al was employed this method for the assessment of diuretic activity. The animals were deprived of food and water for 16tn hours prior to the experiment. Before oral administration of test drug the animals were dosed with 25ml/kg body weight of normal saline. The total volume of was measured. The urinary pH, sodium, potassium and chloride also determined. The diuretic activity result of *EC* was derived and tabulated below.

Table no 37: Effect on urine volume of *Eladhi Chooranam* on various treatment groups on SH-rats

S:NO	Groups	Treatment	Urine volume/100gm/24hr	Diuretic index (24hr interval)
1.	I	Control	7.12±1.04	-
2.	II	Furosemide	14.02±3.02	2.01
3.	III	ECM 200mg/kg	7.21±2.06	2.01
4.	IV	ECM 400mg/kg	10.82±1.14	2.52

Diuretic index = volume of test group/volume of control group

Values are expressed in mean±SD; . P< 0.05; ** P< 0.01; ***P<0.001, compared with control group (Kruskall Wallis and Mann Whitney test)*

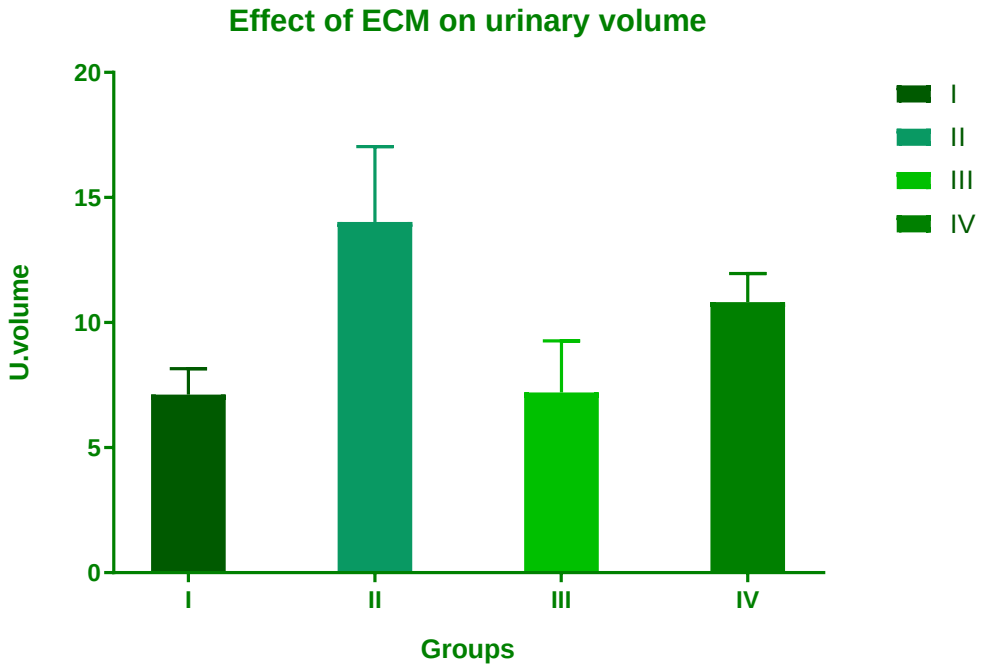


Chart no3: Effect on urine volume of *Eladhi Chooranam* on various treatment groups on SH-rats

Table no 38: Effect on urine electrolyte excretion of *Eladhi Chooranam* on various treatment groups on SH-rats

Groups	Na^+ m.mol/L	K^+ m.mol/L	Cl^- m.mol/L	Na/K
Control	116±2.20	53.2±1.10	89.2±2.14	2.06
Frusemide	138±1.10	92.2±2.20	129.2±2.04	2.32
ECM 200mg/kg	175.1±1.02	100.1±1.04	150.2±1.14	1.62
ECM 400mg/kg	159.03±1.02	76.62±2.31	112.3±2.31	1.02

Values are expressed in mean±SEM; . P< 0.01; ** P< 0.001; *** P < 0.001, compared with control group (Kruskall Wallis and Mann Whitney test)*

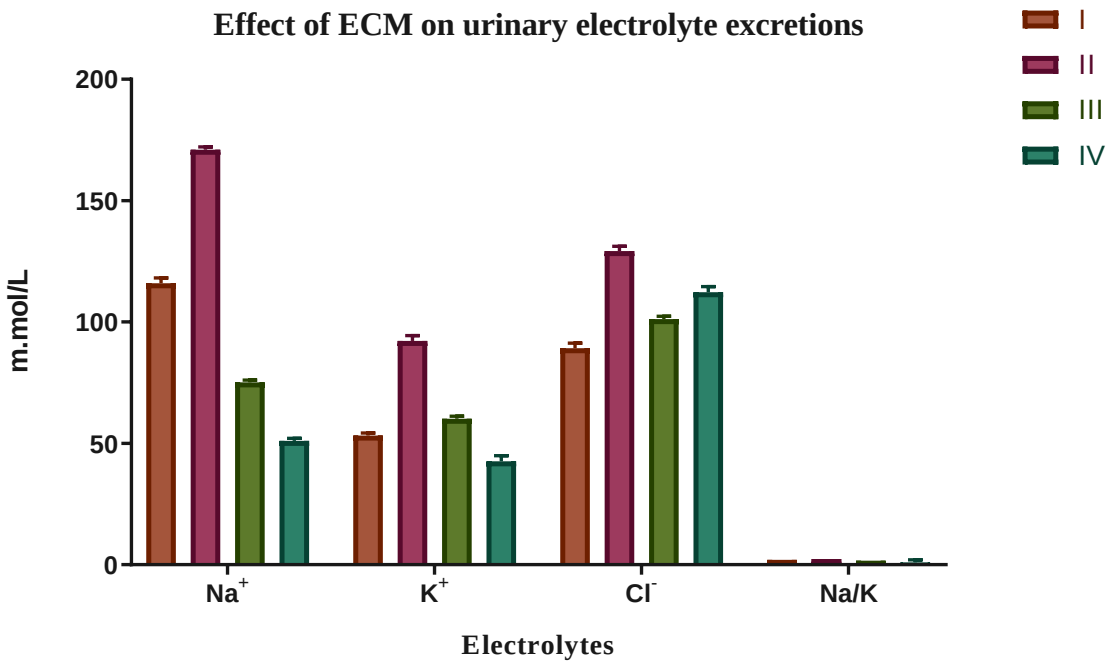


Chart no 4: Effect on urine electrolyte excretion of *Eladhi Chooranam* on various treatment groups on SH-rats

Discussion:

- The results of diuretic activity of *EC* showed marked increase in urine volume
- There was no evidence of dehydration of animals. Animals were observed normal at 5 hours and 24 hours interval.
- The standard diuretic Frusemide significantly increased in urine output when compared to normal
- The test drug *EC* at 200mg/kg b.w, 400mg/kg b.w doses, showed statistically significant increase in the volume of urine with a dose dependent manner
- There is a significant change in the pH level of urine
- Excretion of Na^+, Cl^- followed by similar pattern. Chloride excretion highly significant with two doses.
- The diuretic activity of *EC*, 5 hours after its administration was manifested in the form of an increase in urinary volume, which was highly significant with 200mg/kg b.w, 400mg/kg b.w doses at 5 hours urine analysis
- Analysis of 24 hours post dosing urine sample revealed similar results with regards to urinary volume, sodium, chloride and potassium are observed in 5^{th} hour sample. That indicates a continuation of diuretic effect of *EC* upto 24 hours
- An herbal preparation usually contains many active components (flavanoids, alkaloids, Phenols etc.)
- The phyto chemical analysis of *EC* shows significant presence of these compounds which either alone or in combination is responsible for the diuretic activity
- The diuretic study result of *EC* clearly indicates, that possesses potential diuretic activity on SH-rats and diuretic agents plays an important role in decreasing high blood pressure by decreasing plasma volume and also reducing cardiac work load and the oxygen demand [107] [108]

ANTI-OXIDANT STUDY (In-Vitro)

- Antioxidants are substances that can prevent or slow down to the damage to cells caused by free radicals, unstable molecules that the body produces as a reaction to environmental and other pressures. They are sometimes called "free-radical scavengers."
- Antioxidants are said to help neutralize free radicals in our bodies, and this is thought to enhance the overall health.
- Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress.

Free radicals and their chemical reactions:

- A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals.
- Radicals are weakly attracted to a magnetic field and are said to be paramagnetic. Many radicals are highly reactive and can either donate an electron to or extract an electron from other molecules, therefore behaving as oxidants. As a result of this high reactivity, most radicals have a very short half-life (10^{-6} seconds or less) in biological systems, although some species may survive for much longer.
- Oxidative stress has been linked to heart disease, cancer, arthritis, stroke, respiratory diseases, immune deficiency, emphysema, Parkinson's disease, and other inflammatory or ischemic conditions.

Antioxidant action in Hypertension:

During hypertension, our body went at the condition of oxidative stress. In this oxidative stress condition, oxygen radicals such as superoxide anion, hydroxyl radicals and peroxyl radicals are produced. These oxygen radicals are reactive oxygen species and they can lead to oxidative damage to cellular components such as proteins, lipids, and DNA. These free radicals are reactive molecules involved in many of the physiological process such as atherosclerosis, hypertension and ischemic heart disease. These oxygen free radicals formed during the oxidative stress are

resulting in the disturbances of vasodilators system; particularly degrading of nitric oxide which is mainly due to the endothelial dysfunction are the effects of hypertension [109].

Result:

Table no 39: DPPH assay on *Eladhi chooranam*

Sample concentration (µg/ml)	Absorbance		Percentage of Inhibition	
	Drug	Standard	Drug EC	Standard Ascorbic acid
Control	0.3547	1.7983	0	0.00
12.5	1.0452	1.4044	15.10	21.90
25	0.9818	1.0782	25.44	40.04
50	0.5049	0.7121	52.23	60.40
100	0.1663	0.2921	72.12	83.75
200	0.0827	0.0692	83.68	96.15

**µg/ml: microgram per millilitre. Drug: EC (1.25-20µg/µl). Standard: Ascorbic acid (10mg/ml DMSO)*

Chart no 5.1: DPPH assay on *Eladhi Chooranam* (Percentage of Inhibition)

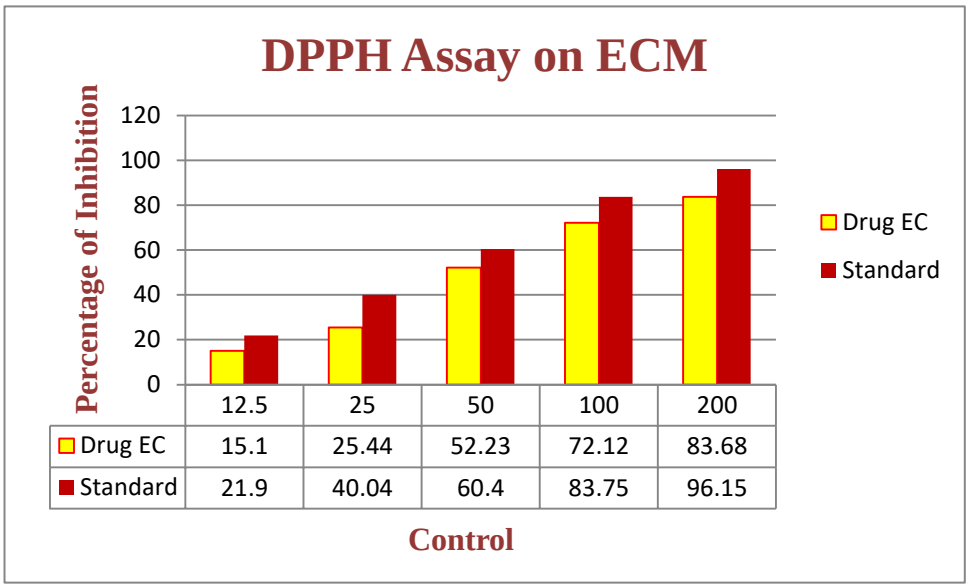
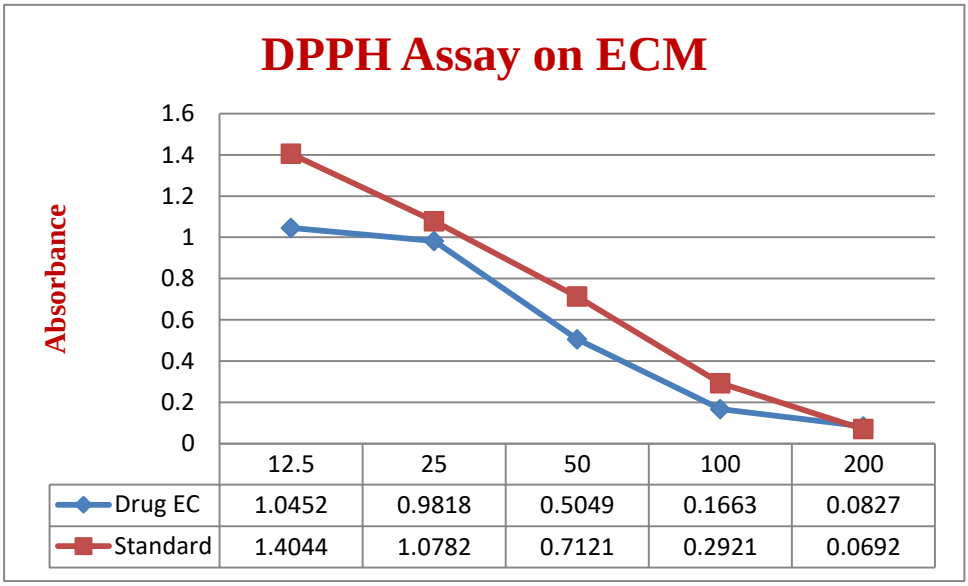


Chart no 5.2 DPPH assay on *Eladhi Chooranam* (Absorbance)



Discussion on Antioxidant activity in DPPH assay:

DPPH assay were used for the determination of Anti-oxidant activity of the different extracts. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of *EC* extract. The antioxidant molecules can quench DPPH free radicals by providing hydrogen atom or by electron donation and a colourless stable molecule 1, 1 diphenyl-2- picrylhydrazil is formed and as a result of which the absorbance at 517nm of the solution is decreased.

In the present study, the *EC* extract was analysed able to decolorize DPPH and the free radical scavenging activity. Ascorbic acid (10 mg/ ml DMSO) was used as a reference and result was expressed as the percentage decrease in absorbance. In the present study, the extract of *EC* was found to possess concentration dependent scavenging activity on DPPH radicals.

The values of DPPH free radical scavenging activity of the *EC* extract was given in (Table) expressed in the percentage. The extract of *EC* was found to possess concentration dependent scavenging activity on DPPH radicals. The extract of *EC* showed the highest DPPH scavenging activity (83.68%) at 200µg/ml and the lowest percentage of inhibition (15.10%) at 12.5µg/ml. Ascorbic acid (Standard) showed highest percentage of inhibition (96.15%) at 200µg/ml and the lowest percentage of inhibition (21.90%) at 12.5µg/ml. This indicated that

% of inhibition increased within increase in concentration of both the standard and *EC* extract. The *EC* extract has more or less equal DPPH scavenging activity when compared to the standard. From the present study, it was concluded that the *EC* extract has a marked antioxidant activity at higher concentrations. Antioxidant compounds are highly present in plants and have protective effects against diseases without reducing their therapeutic efficacy [110]. So, using of natural antioxidant as a protective strategy against cardiovascular related problems [111].

6. CONCLUSION

The intention of this study is to provide a solution for the above need. Eladhi Chooranam was chosen from the Siddha literature as a trial drug ***"Sarabenthirar Vaithya Muraigal "*** written by **Dr.S.Venkatarajan (L.I.M)** to validate the safety and its efficacy of drug to treating Anti-Hypertensive, Diuretic and anti-oxidant activity in Spontaneously Hypertensive Rat (SHR) model.

The trail drug was subjected various studies through which the efficacy of the drug is proved.

The ingredients of the test drug was identified and authenticated by the Siddha experts. The drug was prepared as per the procedure and subjected to various studies to reveal its potency and effectiveness against the disease.

The procedure for drug preparation and its techniques for standardization revealed GMP. The trial drug *EC* has satisfied all parameters of testing protocol for Chooranam which was assigned by AYUSH.

Various analysis such as organoleptic character, physicochemical, phytochemical, biochemical analysis, anti-microbial activity, instrumental analysis were done.

The organoleptic characters of the drug ***Eladhi chooranam*** showed the Brown colour, Bitter amd Sweet in taste which might be responsible for the activity mentioned earlier. The fineness of the *chooranam* represents easy absorption and better availability of the drug.

The physical character of *EC* shows good solubility and the pH of the trial drug is 4.24 that indicate the better absorption and effectiveness.

Phytochemical screening test showed the presence of Alkaloids, Glycosides, Saponin, Phenol, flavonoids, Protein, Diterpines, Gum and Mucilage may be responsible for the anti-hypertensive activity.

The HPLC finger prints were made and it shows 14 peaks. In which the two major peaks denotes presence of phytochemicals and Rf value of the trial drug supports the better standardization of the trial drug *EC.*

Biochemical analysis showed the presence of potassium, calcium, magnesium, ammonium, sodium, chloride and zinc. The potassium, calcium, magnesium, sodium,

chloride supports anti-hypertensive activity and it has a potent anti-oxidant activity of the trial drug *(EC)).*

The anti-microbial activity of trial drug was also considered for its potential.

The instrumental analysis FTIR showed the 9 peak values present, in which the functional groups are alcohol, Phenols, Alkenes, Aldehyde, Carbonyls, Nitro compounds, Carboxylic acids, Esters, Ethers, Amine, Aromatic groups responsible for its activity.

SEM picture described its morphology and the particle size. *EC* which is prepared biologically contains micro particles to enhance the pharmacological action at the target site.

The results of ICPMS shows that there is a mild presence of the heavy metals like As, Cd, Hg, Pb and were below detectable level. This reveals the safety of the drug in treating Hypertension.

Toxicological study of both acute and sub-acute toxicity study were carried out in animal model Wistar albino rat according to the OECD guidelines. The test drug showed no acute toxicity as there was no mortality seen and then 28 days of repeated oral toxicity were done in 28days to show that the trail drug doesn't produce any toxic effect while it was given for long period.

The mortality, functional observations, hematological and biochemical investigations were done. There was no significant change seen in the normal values. Thus the toxicological study of the test drug greatly establishes the safety and gives the justification for long time administration.

The pharmacological study was carried out in the animal model Spontaneously Hypertensive Rats. Three activities were seen in the drug *Eladhi Chooranam*. The activities were

- ✓ Anti-hypertensive activity
- ✓ Diuretic activity
- ✓ Anti-Oxidant activity [IN-VITRO]

Anti-hypertensive activity was carried out in Spontaneously Hypertensive Rats. The trial drug ***Eladhi Chooranam***-400mg/kg b.w showed significant decrease in systolic blood pressure and heart rate. Thus this activity reveals the effect of the drug against Hypertension.

Diuretic activity of ***Eladhi chooranam***-400mg/kg b.w shows statistically increase in urine volume, excretion of urine electrolytes and there is no evidence of dehydration of animals were found, observed normal at 5 hours and 24 hours interval.

Anti-oxidant activity of the test drug ***Eladhi Chooranam*** was carried out in in-vitro model. The *EC* extract has more or less equal DPPH scavenging activity when compared to the standard (ascorbic acid). From the present study, it was concluded that the *EC* extract has a marked antioxidant activity at higher concentrations.

Factors like safety, efficacy, long self like, bio-availability, presence of significant elements; minerals favoring the activity justify the main perspective of this study.

Thus by scrutinizing all the above mentioned factors it is concluded that the trial drug ***Eladhi Chooranam*** is a safe and a potent anti-hypertensive drug. It also possesses diuretic and anti-oxidant activity which supports the effective treatment for managing Hypertension and its complications.

Modern medicine has its own limit in treating Hypertension and controls high blood pressure. Whereas in treating the disease with this trial drug it has a synergistic effect of controlling high blood pressure, diuretic effect and also has anti-oxidant property. Thus it brings a complete treatment for Hypertension and its complications. From the study ***Eladhi Chooranam*** has been proven as a safety and best drug for treating hypertension.

7. SUMMARY

The trial drug ***Eladhi chooranam*** was selected from the siddha literature ***"Sarabenthirar Vaithya Muraigal"*** authored by **Dr.S.Venkatarajan (L.I.M)** for Anti-hypertensive, Diuretic, Anti-oxidant activities**.** The dissertation started with an introduction explaining about the siddha concept, prevalence of hypertension and role of the test drug in treating hypertension,

- The test drug was prepared properly by the given procedure. All the ingredients were identified and authenticated by the experts.
- Review of literature in various categories was carried out. Siddha aspect, botanical aspect and pharmaceutical review disclosed about the drug and the disease. Pharmacological review was done to establish the methodologies.
- The drug was subjected to analysis such as organoleptic characters, physicochemical, phytochemical, biochemical and also instrumental analysis which provided the key ingredients present in the drug thus it accounts the efficacy of the drug
- Toxicological study was made according to OECD guidelines comprising both acute and sub-acute toxicity study. It showed the safety of the drug which attributes its utility in long time administration.
- Pharmacological study was done. It revealed the Anti-hypertensive, Diuretic and Anti-oxidant activity (in –vitro model) of ***Eladhi chooranam*** in spontaneously hypertensive rat model.
- Results and discussion gives the necessary justifications to prove the potency of the drug.
- Conclusion gives a compiled form of the study and explains the synergistic effect of all the key ingredients and activities that supports the study.
- Thus, the Polyherbal formulation ***Eladhi chooranam*** is validated for its safety and efficacy for treating hypertension and it would be a one of the drugs of choice.

8. FUTURE SCOPE

The trial drug ***Eladhi Chooranam*** has its own potency in treating Hypertension in Spontaneously Hypertensive rat model, which has been established in this study. However, the mechanism of action by which ***Eladhi Chooranam*** produced its effect on decreasing the systolic blood pressure and heart rate in experimental animals need to be evaluated in a scientific manner using specific experimental animal models and also multi-center clinical trials are required to understand the exact molecular mechanisms of action. So, it could be used worldwide in treatment of Hypertension.

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Government Siddha Medical College

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CERTIFICATE

Certified that the samples submitted for identification by **Dr.R.Tamilselvan** PG Scholar, Department of *Gunapadam*, Government Siddha Medical College, Arumbakkam, Chennai-600 106, were identified as:

Ingredients of *Eladhi Chooranam*:

1. *Elettaria cardamomum (Elakkai)*
2. *Nelumbo nucifera (Thamarai poovithaz)*
3. *Nymphea nouchali (Alli poovithaz)*
4. *Cyperus rotendus (Korai kizhangu)*
5. *Glycyrrhiza glabra (Athimathuram)*
6. *Syzygium aromaticum (Kirambu)*
7. *Ziziphus marutiania (Ilanthai kottai paruppu)*
8. *Dryobalanops aromatica (Pahai karpooram)*
9. *Oryza sativa (Nerpori)*

Date:

Place: Chennai **PG Department of Gunapadam**

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Approved by Pharmacy Council of India, New Delhi, and
All India Council for Technical Education, New Delhi

L. Uday Metha
Secretary & Correspondent

Dr. Grace Rathnam, M.Pharm, Ph.D
Principal

APPROVAL CERTIFICATE

This is to certify that the project titled "A SCIENTIFIC EVALUATION OF THE POSSIBLE MECHANISMS OF ANTIHYPERTENSIVE, DIURETIC, ANTIOXIDANT ACTIVITTIES OF SIDDHA POLY-HERBAL FORMULATION "***ELADHI CHOORANAM***" IN RODENTS" has been approved by the 53rd IAEC.

IAEC no: 04/321/PO/Re/S/01/CPCSEA dated 12/10/2018

C.L. Baid Metha College of Pharmacy ★ CHENNAI-97 ★ Thoraipakkam

Dr.P.Muralidharan

(Member Secretary)

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY
CHENNAI – 600 119

CENTRE FOR LABORATORY ANIMAL TECHNOLOGY AND RESEARCH

(CPCSEA Approved)

WORKSHOP ON

TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS

CERTIFICATE

This is to certify that Dr./~~Mr./Ms.~~ R. TAMILSELVAN

of Govt. Siddha Medical College, Chennai has participated in the two-day workshop on "TOXICOLOGICAL PROFILING AND ASSESSMENT OF TOXICITY OF DRUGS ON LAB ANIMALS" organized by the Centre for Laboratory Animal Technology and Research, Sathyabama Institute of Science and Technology, Chennai during 31st January – 1st February 2018.

B. Sheela

Chair Person & Coordinator
Dr. B. SHEELA RANI
Director (Research)

Convener
Dr. R. SELVARAJ
Scientist In-charge

The Tamil Nadu Dr.M.G.R.Medical University

69, Anna Salai, Guindy, Chennai - 600 032.

This Certificate is awarded to Dr/~~Mr~~/~~Mrs~~.....R. TAMIL SELVAN.....

For participating as ~~Resource Person~~ / Delegate in the Twenty Fourth Workshop on

"RESEARCH METHODOLOGY & BIOSTATISTICS"

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The Tamil Nadu Dr. M.G.R. Medical University From 24th to 28th April 2017.

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PROF & HEAD DEPT.OF SIDDHA

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of VELUMAILU SIDDHA MEDICAL COLLEGE *attended the National Seminar on "Recent Trends in Biomedical Research" Conducted on 30th october 2015, at VS Clinical Research & Hospitals, TICEL Biopark, Chennai, Tamilnadu.*

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.....GOVT. SIDDHA MEDICAL COLLEGE, CHENNAI.....
*attended the National Conference on "**Herbal Medicine and Ethnopharmacology**" conducted in V.S. Clinical Research & Hospitals (P) Ltd., Chennai, Tamil Nadu. He/She presented a paper/poster in the topic.....*

T. Mathangi

Dr. T. Mathangi
Scientist & Coordinator

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Dr. P. AGASTIAN
CONVENOR, BTDCTC-2019
Dept. of Plant Biology & Biotechnology

Rev. Dr. F. ANDREW, S.J
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